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INTRACELLULAR ACIDITY IN VALONIA.

By W. J. CROZIER.

(*Contributions from the Bermuda Biological Station for Research, No. 105, and from the Physiological Laboratory, College of Medicine, University of Illinois.*)

(Received for publication, April 28, 1919.)

The very large cells of the marine green alga *Valonia macrophysa* permit one to obtain several cubic centimeters of fluid from the central vacuole of a single cell (cf. Wodehouse, 1917), without involving appreciable alteration in the composition of the cell-sap through protoplasmic injury during the process of extraction (cf. Chambers, 1917). Estimations have been made of the acidity of this cell-sap. These measurements, although perhaps revealing no essentially new points of theoretic interest, are nevertheless valuable, since they bear so directly upon some questions regarding the reaction of the cell interior.

The cell-sap of *Valonia* is much more acid than the sea water in which the plant is living. The reaction of the sap from fifty cells as obtained immediately after their removal from the sea was found to vary in individual cases from pH 5.0 to 6.7, the mode being at 6.0, the average 5.9. In thus possessing an acid internal medium the *Valonia* cells resemble those of some flowering plants containing natural indicators (Haas, 1916).

Valonia grows in great masses at a depth of several feet beneath low-water level in certain mangrove "creeks," and also, less abundantly, among other seaweeds, on the reefs. The alkalinity of the sea water in the tidal creeks depends upon the state of the tide: at flood-tide, for example, in Fairyland Creek, the water most distant from the mouth of the creek was at pH = 8.2, while that half way toward the mouth of the creek was at 8.1, and in the adjacent regions of Great Sound, 8.07; at low-tide next morning, the alkalinity at the head of the creek was pH = 8.3. A similar rhythmic altera-

tion of alkalinity, conditioned by photosynthesis, and by the pulsatile supply of "outside" water to the creek at periods of high-tide, was regularly observed. Over the reefs a less extensive daily rhythm of variation in alkalinity was noted (cf. McClendon, 1918), the reaction of the ocean water being at pH = 8.1 to 8.2; the variation here concerned depended, in part, upon the ebbing of water from semi-enclosed sounds.

The acidity of the sap of *Valonia* under these differing external conditions was found, however, to be sensibly the same in all cases, even when the temperature varied from 18–28°C., at different times of the year.

No effect of darkness (*i.e.* cessation of photosynthesis) could be detected in the sap reaction, either in the field or when the cells were kept in darkened aquaria for some hours.

For the field measurements in particular, it was necessary to use various indicators in connection with buffer solutions. The sap to be tested was secured by first rinsing a selected cell rapidly in distilled water, drying it with filter paper or a soft cloth, then puncturing the cell-wall with a glass needle, thus allowing the sap to flow out into a test-tube. The addition of 1 to 2 cc. of sea water to 10 cc. of sap is sufficient to change the pH from 5.9 to 7.0,—hence contamination with sea water had to be avoided. Carbon dioxide, moreover, is readily lost by the *Valonia* sap, in which it exists at a considerable tension, so that squirting the sap through the air from a small puncture in the cell was not permissible.

In aquaria, *Valonia* quickly increases the alkalinity, through photosynthetic abstraction of CO_2, and can in this way bring about an external alkalinity of pH = 9.5, or even greater (cf. Moore and others, 1914; Crozier, 1919). But the *internal* acidity seems to be constantly maintained, even under these extreme conditions, at a little less than pH = 6.0. The same was found in moderately hypo-alkaline sea water (pH = 7.8 to 7.9). This finding indicates that in a method of studying photosynthesis, for example, such as that devised by Osterhout and Haas (1918), the varying external reaction forming the basis of the measurements does not itself materially affect the internal acidity, the latter presumably being the immediate regulator of respiratory and other protoplasmic processes.

At the same time, it seemed from my estimations highly probable that the reaction of the sap from different healthy cells was not always the same, even with careful precautions in taking the readings. No correlation could be seen between the size of a cell and the reaction of its fluid contents—the sap from young, actively growing cells was not more alkaline than that from much larger, healthy cells.

It was shown by Wodehouse (1917) that sap from dead *Valonia* cells contained SO_4, while that from healthy cells did not; occasional cells, otherwise healthy in appearance, showed a trace of SO_4 when tested with $BaCl_2$. In many instances I found an acidity of pH = 7.0 or less (7.0 to 8.0), always accompanied by the presence of SO_4. Cells obviously disorganized, though only to a slight degree, were always in this category. During 3 years several hundred cells in all were tested. As in some other plant cells (Haas, 1916), the acidity of the vacuole content decreases as natural death approaches; the reverse occurs in at least some plant cells, and in an animal, *Chromodoris* (Crozier, 1918 *b*). In *Valonia* it has been clear that natural death of a cell consumes some time; the change in the acidity of the cell-sap, paralleled by the change in permeability to SO_4, being a gradual process (cf. Osterhout, 1917).

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ON THE CONTROL OF THE RESPONSE TO SHADING IN THE BRANCHIÆ OF CHROMODORIS.

By W. J. CROZIER.

(*Contributions from the Bermuda Biological Station for Research, No. 106, and from the Physiological Laboratory, College of Medicine, University of Illinois.*)

(Received for publication, April 28, 1919.)

The gill-plumes (branchiæ) of the nudibranch *Chromodoris zebra* are normally retracted within the branchial collar after a shadow is cast on the animal (see Fig. 1). If the decreased light intensity persists, the gill-plumes become reextended, but usually not so fully as in the first instance. Characteristically, in the dark the branchiæ are concealed within their collared pocket, and in the light become extended to a degree depending on the light intensity. It has been shown (Crozier and Arey, 1919) that the photic control of gill-plume extension depends upon the activation of photoreceptors located within, and in the immediate vicinity of, the plumes themselves, and not upon the eyes of the nudibranch. The mechanism of this control is complex: (1) the integument in the region of the branchial collar is photosensitive and when it is illuminated induces the protrusion of the branchiæ; (2) the branchiæ themselves, through the action of a self-contained nerve net, respond by contraction when the intensity of the incident light is quickly decreased, but do not react to an increase in light intensity; (3) if a sufficient number of the gill-plumes is excited by shading, their individual contractions reflexly determine a retraction of the whole gill-crown, together with the sphincterwise closure of the branchial collar. These responses have nothing to do, directly, with the positive phototropism of the nudibranch, although, as Arey and I (1919) have intimated, it is significant that the locomotive activities initiated by light in a previously darkened and quiescent *Chromodoris* are correlated with the protrusion of the chief respiratory organs.

It was desirable to consider some of the influences controlling the variability in the responses of the gill-plumes when shaded; for, as noted in the paper referred to above, the exhibition of the reaction is by no means constant. In nature *Chromodoris* shows but slight variability with regard to the amplitude of the response to shading. With animals of medium size (6 to 10 cm. long), at about 27°, an interval of 10 to 15 minutes must elapse between successive stimula-

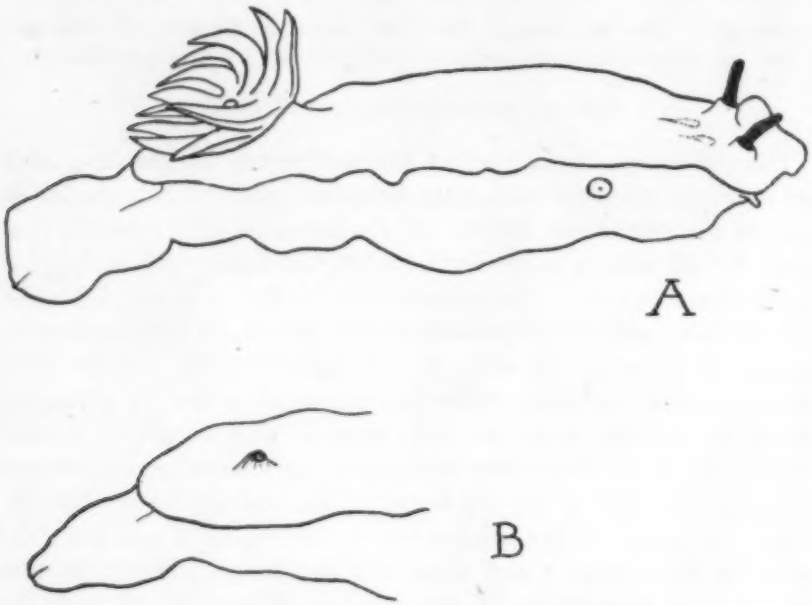

FIG. 1. A. Semidiagrammatic view of *Chromodoris zebra*, showing the gill-crown normally extended. The number of plumes varies from eight to sixteen, with a mode of twelve.

B. The gill-crown contracted within its pocket, the collar closed.

tions in order to obtain the maximal reaction at each trial. The great variation apparent in laboratory tests is determined by the confinement of the nudibranchs to aquaria. In dishes of non-circulating water the alkalinity is slowly reduced, owing to the excretion of CO_2. The nature of the response to shading was determined in a series of twenty shallow vessels of similar size and shape, exposed to the same conditions of illumination, in which the alkalinity had been

reduced to varying degrees. The responses of each individual nudibranch (forty-five in all) were observed under conditions of gradually diminishing alkalinity.

The results of these experiments were uniform. At an acidity of pH = 8.0 the reaction when the animal was shaded, both in light from a north window and in bright sunlight, was entirely normal as to amplitude, but the latent interval preceding the response was perceptibly lessened; at pH = 8.2 (about the natural alkalinity) the mean latent interval, measured with a stop-watch, was 1.0 second at 28°; at pH = 8.0 it was 0.7 second. With still lower alkalinities the following series of events was made out.

pH of sea water.	Nature of the response to shading (at 28°C.).
8.2	Normal. Latent period 1.0 sec.
8.0	Amplitude normal. Latent period slightly abbreviated (0.7 sec.).
7.95	Latent period lengthened (2.0 to 3.0 sec.). The ensuing reaction usually violent and complete.
7.9 to 7.8	A delayed response, like the preceding, but in less than half the animals tested. Sometimes no response. Occasionally a plume or two was seen to react. Branchiæ much extended, even in the dark.
7.7 to 7.6	No response whatever under any conditions of illumination. Gill-crown greatly extended (Fig. 2). The branchiæ still reactive to tactile stimulation, but merely by individual contraction (curling up). Impossible to cause the retraction of the whole branchial crown.

Acid (HCl) was added to sea water until the pH was reduced to 6.3, and in this medium *Chromodoris* lived for an hour or more in a closed vessel. The gill-crown, as at pH = 7.6 to 7.7, was much protruded (Fig. 2).

Alkalinities greater than normal, obtained by allowing algæ to remain in the dishes of sea water for an hour or more and also by additions of alkali (NaOH), were found to result in a lessened protrusion of the gill-crown. Whereas in diffuse light the gill-plumes were extended to a moderate degree at pH = 8.2, the whole branchial apparatus became retracted and concealed from view at pH = 8.4. At an alkalinity of pH = 8.4 the plumes could not be made to extend by the incidence of direct sunlight. The effect of hyperalkalinity upon the exhibition of a reaction to shading is thus in part a secondary

matter, since—the gill-crown being as a whole less extended, and its individual plumes less turgid—the direct responses to shading (upon the magnitude of which the reflex contraction of the whole crown is dependent) are specifically less vigorous. But it seemed clear that the photic irritability of the intrinsic reacting mechanism of the plumes was also less than in water of pH = 8.2; this was tested by comparing the reactions of individual plumes shaded singly.

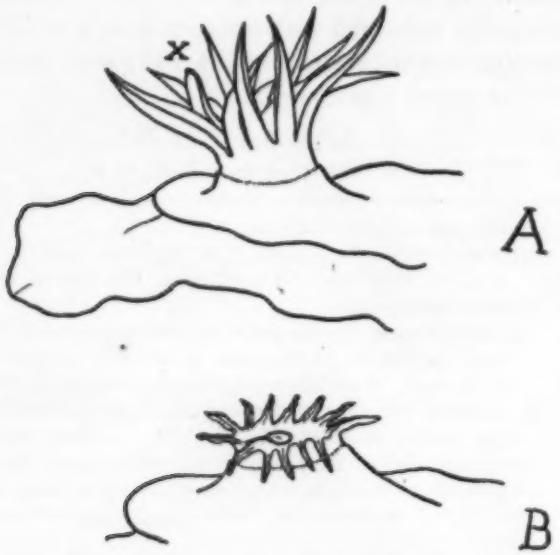

FIG. 2. A shows the great protrusion of the gills as seen at high temperature, in direct sunlight, and especially in sea water of diminished alkalinity; at *x*, an injured plume.

B shows how the retraction of the branchial crown normally resulting from the contraction of the plumes is inhibited at low alkalinities of the sea water; the plumes are here sketched as contracted maximally by touching them sharply.

Two other influences controlling the exhibition of a response to shading in *Chromodoris* are temperature and light intensity. Supranormal temperatures cause the branchiæ to become maximally protruded; this is seen when the nudibranchs are immersed in water at temperatures of 32–35°. With the sea water cooled to 10°, the branchiæ become much retracted; at temperatures between 12 and 17°, the photic reactivity of the branchiæ is much reduced. Direct

sunlight causes the gill-crown to be very fully expanded, and after a short time (15 minutes) the branchiæ react very feebly if at all to shading. At 30.5°, no shading response was obtainable in direct sunlight, but there was a slight one in diffuse daylight.

Thus the branchiæ of *Chromodoris* are extended under those conditions which also normally determine an increased neuromuscular activity, creeping. The gill protrusion commences, however, in the case of light, before the increased creeping activity is well started; therefore the heightened activity is not the cause of the gill protrusion. Moreover, as already stated, the control of the gill-plumes and of the gill-crown as a whole is exercised by a mechanism locally contained, while the photokinetic movements are initiated probably through the eyes. Conversely, low temperatures, darkness, and slightly increased alkalinity, which lead to a decrease in motor activity, cause the plumes to be retracted to a greater or less degree.

Only when the protrusion of the plumes is permitted by the temperature and light conditions is the reaction to shading possible.

The response is further controlled by the reaction of the medium, being possible only within the range pH = 7.8 + and 8.3 ±, the normal range of alkalinity in the habitat of this animal being 7.9 + to 8.25.

At the acid end of this range the intrinsic photic irritability of the plumes is much decreased, or inhibited, and at a slightly higher acidity the reflex retraction of the gill crown is inhibited. The "advantageous" features of this state of affairs may be noted: when the CO_2 content of the water is greatly increased, the gills become widely spread and no longer interrupt their respiratory function by retracting when shaded; whereas, if the alkalinity of the water is increased (correlated, under natural circumstances, with increased O_2 concentration), the gill-plumes—"no longer so much needed"—remain concealed within their collared pocket.[1]

Locomotor activity, protrusion of the gills, and production of CO_2 continue in water of an alkalinity so low (pH = 7.9) that free

[1] In numerous other instances where animals react to shading the response is characterized by great variability; this type of response should therefore provide a good opportunity for quantitative study. Possibly in other instances also some of the controlling influences here distinguished may be found operative.

oxygen no longer exists in the medium (McClendon 1917, p. 34); in *Cassiopea*, according to Carey (1917), pulsations of the swimming bell cease in sea water less alkaline than pH = 7.9, but *Chromodoris* moves about for a time in solutions more acid than this.

The control of gill protrusion, and of the photic irritability of the plumes by the existing alkalinity, which is prominent at the extremes of the normal range, is probably exercised, to a less conspicuous degree, under the normal variations of loss of CO_2 from the body of the nudibranch. The whole system of gill reactions is apparently concerned fundamentally with regulating the loss of CO_2 from the blood, analagous to the method whereby the respiratory rhythm of a vertebrate is controlled, but with this difference: the diffusion of CO_2 from the respiratory surfaces is directly controlled in *Chromodoris* by varying the amount of surface in contact with the external medium. Upon this respiratory control of the gill movements the property of branchial contraction as the result of shading is superimposed; it is a method of response which is of secondary importance. In order that the shading reaction may be exhibited in its full amplitude, the gill-crown must be at a certain stage of extension, while the intrinsic reactivity of the individual plumes is also limited by at least one of the factors which determine the degree of gill-crown protrusion. The sensitivity of the plumes to a decrease in light intensity is probably of some protective value. The gills are bitten at by fishes, and in about 10 per cent of the individuals the plumes are found to have endured some degree of injury, presumably from this cause (cf. Crozier, 1919). Hence it is of interest that the protective aspect of the branchial activity should clearly be a secondary thing, in spite of the fact that injury is continually being suffered by the gills. It is entirely possible that under natural conditions the photic irritability of the plumes should be a function of the reaction either of the sea water or of the blood of the nudibranch. It has not been possible, however, to discover any relation between the irritability of the gills in this respect and the tint of the indicator pigment in the epithelium of the gills; in different animals this tint is blue, purple, or even pinkish, possibly indicating variations in intracellular acidity (the indicator

turns bright pink at pH = 5.6 ±; cf. Crozier, 1916); but the color of the gills is independent of that of the other tissues of the animal, although due to the same pigment.

SUMMARY.

The branchial plumes of *Chromodoris* respond, by contraction, to a decrease in light intensity. This response is obliterated by high temperatures (above 32°) and by direct sunlight, and is possible only within a limited range of alkalinity of the sea water. A concealing retraction of the whole gill-crown is reflexly determined by the self-contraction of the individual plumes under "optimal" conditions of light, temperature, and alkalinity. This protective response of the branchiæ is superimposed upon their simple system of fundamental activities (protrusion, retraction) apparently concerned with regulating the respiratory exchange of the nudibranch.

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IODINE AND THE THYROID.

III. The Specific Action of Iodine in Accelerating Amphibian Metamorphosis.

By W. W. SWINGLE.

(*From the Department of Biology, Princeton University, Princeton.*)

(Received for publication, May 13, 1919.)

In previous studies on the relation of iodine to the thyroid, as determined by the effects produced by feeding this substance and its compounds to larval Anurans,[1] the following conclusions were either stated or implied, all of which have a direct bearing on the present experiments. (1) Inorganic iodine and its compounds, iodoform and potassium iodide, greatly accelerate metamorphosis of tadpoles. (2) Animals from which the thyroid gland had been removed at its inception (*i.e.* 6 mm. larvæ), and which under normal conditions never undergo metamorphosis but grow to an abnormal size, quickly transform into frogs when fed iodine. (3) The follicles of the thyroids of tadpoles on an iodine diet show a greater colloid content than do the glands of normally fed animals. These facts led to the conclusion that iodine is essential for amphibian metamorphosis, that it is the active constituent of the thyroid glands of these animals, and, judging by its action on thyroidless tadpoles, that it exerts its action directly upon the cells and tissues of the organism without the necessity of undergoing transformation in the gland tissue; *i.e.*, that iodine is capable of functioning as the thyroid hormone itself within the body, or else is transformed into this hormone through the activity of tissue other than that of the thyroid. (4) The chief function of the thyroid is the extraction from the blood and storage of the minute quantities of iodine taken into the organism in food and water, and the subsequent release of this sub-

[1] Swingle, W. W., Studies on the relation of iodin to the thyroid, *J. Exp. Zool.*, 1918–19, xxvii, 397, 417.

stance into the general circulation under pressure of the organism's needs.

In the experiments recorded here, further evidence, indicating that the conclusions stated are essentially correct, is presented.

The problem, with which the present experiments are concerned, was suggested by Professor E. Newton Harvey, in whose laboratory the work was done, and to whom the writer is indebted for suggestions, equipment, and criticism. Briefly stated, the problem was to determine if the action of iodine upon Anuran metamorphosis is specific, or if other closely related chemical substances, such as bromine, exert a similar effect; to determine by quantitative feeding the amount of iodine, or other substances if any were found, required to produce metamorphic changes in tadpoles kept under uniform conditions; and finally to compare the histological picture presented by the thyroids of the iodine, bromine, and normally fed animals.

Throughout the work, unless otherwise stated, the larvæ used were *Rana sylvatica* LeConte. All animals came from the same egg mass, and hence were of the same age. They were permitted to develop to a total length of 10 mm. before being used, though no food was fed them during this interval, nor was any needed as the larvæ subsist and increase in size by utilization of the yolk in the body cells.

The food given consisted entirely of algæ, practically all gathered from the same pool, and *Elodea* from the University vivarium, fed in equal quantities. The only animal matter the tadpoles received was that entangled in, or living on, the algæ. The environmental factors of temperature, light, and water supply were kept as nearly uniform as possible. Too much stress cannot be laid upon these factors in any work concerned with growth and metamorphosis, as the extreme variability often observed in the growth rates of frog cultures is due largely, in the writer's experience, to slight disturbances of these environmental factors.

Each culture consisted of thirty-two larvæ in 2,000 cc. of ordinary tap-water, with fairly high calcium content. The water was changed daily.

Iodine and bromine were made up in chemically equivalent stock solutions of M/10 concentration, the iodine in alcoholic, the bromine in aqueous, solution.

The fact that the iodine was in alcoholic and the bromine in aqueous solution had no effect on the results obtained. This is clearly shown elsewhere where similar results were obtained and an alcoholic solution was not used.[1] Five cultures of larvæ received gradually increasing amounts of iodine and bromine. Thus Bromine Culture 1 received $\frac{1}{23}$ cc. of M/10 stock solution of bromine in 2,000 cc. of H_2O, yielding M/460,000 solution. Iodine Culture 1 received $\frac{1}{55}$ cc. of stock solution iodine in 2,000 cc. of H_2O, making M/1,100,000 solution. More drops of the alcoholic solution are required to make 1 cc. than of the aqueous solution due to the difference in surface tension of the two solutions, hence the difference in the size of the drops. Cultures 2, 3, 4, and 5 of the bromine set received respectively $\frac{3}{23}$, $\frac{4}{23}$, $\frac{5}{23}$, and $\frac{7}{23}$ cc. of the M/10 solution. Cultures 2, 3, 4, and 5 of the iodine set received respectively $\frac{3}{55}$, $\frac{4}{55}$, $\frac{5}{55}$ and $\frac{7}{55}$ of the M/10 solution iodine. Bromine Culture 2 compares, in regard to strength of solution used, with Iodine Culture 4, though the iodine culture is considerably the weaker. An exact chemical equivalent solution falls somewhere between Bromine Cultures 1 and 2 and Iodine Culture 4.

It is probably not necessary that iodine or bromine obtain entrance to the organism through the alimentary tract; they will probably be absorbed and reach the vascular system almost as rapidly through the integument.[2]

Observations.

Experiment 1. Iodine Feeding.—The tadpoles, fasting since their emergence from the egg jelly, measured 10 mm. in length, and showed no indications of limb buds when first fed iodine. There were six cultures, one serving as control for the other five. There was no change during the first 32 hours in Cultures 1, 2, and 3, some mortality in Cultures 4 and 5, especially in 5. At the end of 60 hours the larvæ in Cultures 4 and 5 showed, on microscopic examination, considerable fraying of the delicate tail-fin and extravasation of blood

[2] McClure, C. F. W., On the behavior of *Bufo* and *Rana* toward colloidal dyes of the acid azo group, American Anatomical Memoirs, No. 8, 1918. Swingle, W. W., On the experimental production of edema by nephrectomy, *J. Gen. Physiol.*, 1918–19, i, 509.

from the capillary vessels. Ten animals in Culture 5 had died, two in Culture 4, none in the remainder of the cultures.

86 hours after the first iodine was fed the larvæ were again examined. All of the iodine-fed animals were more deeply pigmented than the controls. Cultures 1 and 2 showed practically no tail shrinkage, whereas this showed clearly in Cultures 3, 4, and 5. The tadpoles in the stronger solutions also had well developed epithelial hind limb buds. In the controls and in Cultures 1 and 2 only the largest animals revealed any indications of such limb anlage. As is well known, the hind limbs of the Anura develop from the epithelium, and in the writer's experience, appear very early; but they remain mere formless buds for some time, usually until the animal has attained a length of 24 or 25 mm. and sometimes even larger. The limb buds then develop the two primary leg divisions and later the toes differentiate. Iodine feeding causes rapid development of the limb rudiments.

March 29, 1919. 9 days from date of first feeding. Controls: average total length 13.5 mm. Hind limb buds not visible without the aid of the microscope. Pigmentation normal. Animals active and well nourished.

Culture 1.—Average length 13 mm. Hind limb buds but slightly larger than those of the controls, pigmentation normal. Animals well nourished.

Culture 2.—Average length 13.5 mm. Hind limb buds large. Easily visible without microscope; some measured over 1 mm. in length. Pigmentation dark Slight emaciation of the body and some shrinkage of the tail-fin. It was observed for the first time on this date that the tadpoles in all cultures except No. 1 remain near the surface of the water. This continued throughout the experiment, the animals descending to the bottom of the container only to obtain food.

Culture 3.—Average length 12 mm. Hind limb buds now differentiating into tiny legs. Pigmentation dark. Emaciation slight. Considerable atrophy of the tail-fin. Animals sluggish.

Culture 4.—Average length 12 mm. Tiny hind legs with toes. Pigmentation dark. Animals sluggish. Tail-fin atrophied. Slight emaciation.

Culture 5.—Average length 12 mm. Tiny hind legs present. Great emaciation and tail shrinkage present. Animals darkly pigmented. Considerable mortality.

April 4, 1919. Controls: average length 14.5 mm. Hind limb buds just visible without the microscope in animals over 15 mm. in length.

Culture 1.—Length 14.5 mm. Identical in development with the controls except for slightly larger limb buds.

Culture 2.—Length 15 mm. Hind limbs larger than those of animals in Culture 1. Pigmentation dark. Previous emaciation has disappeared. Atrophy of tail-fin gradually disappearing.

Culture 3.—Length 13 mm. Limbs larger and better developed than those of animals in Cultures 1 and 2. Slight emaciation present, also tail-fin atrophy. Pigmentation lighter than controls.

Culture 4.—Length 13.5 mm. Limbs large and well developed. Some emaciation. Tail-fin atrophied. Animals sluggish. Pigmentation dark.

Culture 5.—Abandoned on this date. The mortality rate was too high to continue the experiment. It was observed that though the death rate was high during the first week of iodine feeding in quantities such as were given here, the animals which survived could stand double the dosage given previously.

April 13, 1919. Controls: Length 21 mm. Hind limbs tiny epithelial buds with no differentiation into primary divisions or toes.

Culture 1.—Length 21.5 mm. Hind limbs larger and better differentiated than controls. Otherwise no difference in the animals of the two cultures.

Culture 2.—Length 22 mm. Hind limbs fully differentiated. No emaciation. No tail-fin atrophy, the atrophy apparent on previous examination had disappeared and did not occur again. Pigmentation dark. Animals remarkably uniform in size, something rather unusual in a culture of frog larvæ.

Culture 3.—Length 18.5 mm. Legs fully formed and large. Pigmentation lighter than controls. Slight emaciation and tail shrinkage present. Animals sluggish.

Culture 4.—Length 19.5 mm. Legs large for body, and well formed, average 6 mm. in length. Pigmentation dark. Tail shrinkage apparent. When examined under the microscope, the fore limbs were found pressing against the skin of the pectoral region where later they burst through.

It will be noted that the tadpoles were increasing rapidly in size. Until this date, the controls were the largest animals, the iodine cultures varying but slightly in this respect. It is interesting to note that larvæ of a similar age, left to develop under more natural conditions than prevail in the laboratory, had just hatched, and were not over 7.5 mm. long. At the time the eggs were collected, some of them were placed in a small, deep pool of clear water, plentifully stocked with food, in a sheltered spot not far from the laboratory, where their development was closely followed. The laboratory animals averaged over 20 mm. in length before the larvæ in the pool hatched. This difference in growth rate was due to the uniform heat of the laboratory, and to the unseasonably chilly weather.

April 20, 1919. Controls: Length 25 mm. Limbs tiny epithelial buds as yet undifferentiated.

Culture 1.—Length 26 mm. Legs small but perfectly developed. Pigmentation dark. Animals well nourished.

Culture 2.—Length 29 mm. Very large, well nourished animals. Limbs large. Fore limbs just visible under the skin. Pigmentation dark.

Culture 3.—Length 23 mm. Legs 7 mm. long. Pigmentation light. Animals very sluggish. Fore limbs easily visible under the skin.

Culture 4.—Length 26 mm. Hind legs 9 to 10 mm. long. Tail shrinkage apparent. Fore legs through skin on side in three animals. Pigmentation dark. Animals sluggish.

On this date the larvæ left in the pool were examined again. The average length was 13.5 mm. No limb buds were visible. Pigmentation slightly lighter than that of the laboratory controls.

April 27, 1919. Controls: Length 26 mm. Otherwise no change.

Culture 1.—Length 28 mm. Limbs larger and better developed than those of controls.

Culture 2.—Length 31 mm. Legs well developed otherwise no change.

Culture 3.—Length 23 mm. No change, except an increase in the size of the legs.

Culture 4.—Length 27 mm. Fore legs through skin in several animals. Hind legs very large.

Animals in the pool were again measured. The average length was 15 mm. Tiny epithelial hind limb buds present. No differentiation yet. Lightly pigmented. Animals well nourished and active. The experiment was abandoned May 1, 1 month and 10 days from the date of the first iodine feeding.[3]

Discussion of Experiment 1.

Metamorphosis.—The effects upon metamorphosis of feeding known quantities of iodine in progressively stronger concentrations is obvious from this experiment and needs only brief mention here. It is clearly shown that the rate of metamorphic change in Anuran larvæ depends upon the amount of iodine the animals receive; the stronger the concentration, the more rapid the change from larval to adult condition. Iodine even in such minute quantities as Culture 1

[3] Though no iodine was fed after May 1, and but little food given all the animals of Cultures 3 and 4 completely metamorphosed by May 17. Seven larvæ of Culture 2 had transformed by June 1. Two animals from Culture 1 metamorphosed June 10. None of the control tadpoles has yet completed metamorphosis.

received (M/1,100,000 solution) soon showed the effect of its administration. It is safe to assume that an absolutely iodine-free diet fed to frog larvæ reared in iodine-free water would inhibit metamorphosis indefinitely. The writer is conducting experiments along this line, but the data are not yet ready for publication. As stated elsewhere, the function of the thyroid appears to be that of collecting, storing, and passing into the circulation at the proper time such iodine as finds its way into the organism by means of food and water. Probably most if not all organic matter contains very minute traces of iodine in some form; most surface water contains traces of it, though iodine is said not to occur in deep fresh water springs. Lack of iodine in the environment may possibly explain why *Amblystoma trigrinum* fails to undergo metamorphosis and permanently retains its larval form in certain localities such as Lakes Xochimilco and Chalco 8 miles southeast of the City of Mexico.[4] This phenomenon is not unknown elsewhere; for instance, in certain pools and districts in Lombardy the larvæ of Triton are said to fail to metamorphose and may grow to an immense size. It seems reasonable to suppose, in the light of these iodine studies, that the failure of the Axolotl and Triton larvæ to metamorphose is due to lack of iodine in the environment or else the thyroids of these animals are defective and unable to collect sufficient iodine for the organism's needs. Just as there are certain districts where cretinism and myxedema are endemic, so there are certain districts where amphibians fail to undergo metamorphosis. In both instances lack of sufficient iodine in the food and water is probably the causal factor.

Moreover, it seems likely that in the experiments recorded in the literature where frog larvæ restricted to certain diets, for example thymus gland tissue, apparently fail to undergo metamorphosis at the proper time, or not at all, the cause of the delayed metamorphosis is lack of sufficient iodine. Thymus tissue is said not to contain iodine, but in the mammalia it is not at all uncommon to find thyroid tissue embedded in the thymus. The discordant results obtained by investigators who have fed thymus tissue to frog larvæ may be explained on this hypothesis, although the writer believes that certain

[4] Gadow, H., Through Southern Mexico, London, 1910.

other environmental factors also play a part which may lead to misinterpretation of results.[5]

In a recent number of this *Journal*, Uhlenhuth, while accepting the conclusions stated regarding the relation of iodine to amphibian metamorphosis, thinks that still another substance is needed to cause the thyroid gland to excrete the iodine necessary for metamorphosis. This hypothetical factor he terms an excretor substance[6] and thinks that it is evolved during the growth processes of the organism.

The assumption of an excretor substance obscures rather than clarifies the already sufficiently complicated problem of amphibian metamorphosis by the introduction of an unknown and purely hypothetical agent. That iodine is essential for metamorphosis we have seen, but it is equally certain that iodine is not the sole factor involved, or else Anuran larvæ of the different species, having different periods of larval life, should if reared together and fed equal amounts of iodine undergo metamorphosis simultaneously. This they will not do. The writer believes that the long larval life of *Rana catesbiana* is related to slow thyroid development in that species and the consequent inability of the organism to utilize iodine except in large amounts; conversely, that the short larval life of *Bufo* is due to very rapid thyroid differentiation, hence greater powers of iodine assimilation. There is no experimental proof to substantiate this view and it is to be regarded merely as a suggestion.

Amphibian metamorphosis is an extremely complicated phenomenon, and in the last analysis will probably be found to depend, not on a single factor, but on a group of factors both extrinsic and intrinsic, the cooperation of which is necessary to complete the process.

Growth.—The data on growth recorded for the iodine-fed tadpoles showed that the size of the larvæ varied considerably for each individual culture; at a given time some of the animals would be 10 to 12 mm. longer than others of the same culture, but for the experiment as a whole there was a continuous upward trend of the growth curve

[5] Gudernatsch, J. F., Feeding experiments with tadpoles. II, *Am. J. Anat.*, 1913–14, xv, 431. Swingle, W. W., Experiments with feeding thymus glands to frog larvæ, *Biol. Bull.*, 1917, xxxiii, 116.

[6] Uhlenhuth, E., Relation between thyroid gland, metamorphosis, and growth *J. Gen. Physiol.*, 1918–19, i, 473.

of the animals in each of the cultures. There was no continuous upward or downward growth curve depending upon the iodine concentration. Thus, the animals of Culture 2 outgrew both controls and the animals in stronger concentrations; the animals in Culture 3 were smaller than those of Cultures 2 or 4. Strong concentrations of iodine bring about cessation of growth very quickly, and differentiation is stimulated, whereas very weak solutions such as those of Cultures 1 and 2 tend to stimulate both growth and differentiation. Rapid differentiation apparently does not occur along with rapid growth; an overdose of thyroid extract or iodine to frog larvæ immediately starts the mechanism of differentiation. If the larvæ are very young, not only does growth cease, but differentiation does not proceed very far before death of the organism supervenes due to the extreme height to which metabolic activities rise. The organism literally withers away.

The work of Janney is of interest in this connection.[7] He has shown that an overdose of the thyroid hormone leads to a loss in weight and size of the organism; conversely, a certain minimum dose results in a gain in both size and weight. This is very well shown in the present experiment, the animals of Cultures 1 and 2, receiving minimum doses of iodine, outgrew the controls and the animals of the stronger concentrations. As shown elsewhere[1,8] by the writer, overdosage with iodine or thyroid extract leads to decrease in size and great emaciation of the animals. The conclusion in regard to growth is that iodine in minimum doses stimulates growth, though not to any great degree, and in larger doses it leads to cessation of growth and tissue disintegration or absorption. Iodine feeding has but little effect on pigmentation. If fed in sufficiently strong concentrations the animals appear darker than the controls.

Experiment 2. Bromine Feeding.—The results obtained by feeding iodine did not, however, rule out the possibility that the accel-

[7] Janney, N. W., III. Studies in thyroid therapy. The effect of the thyroid hormone as determined by a clinical metabolic and dietetic investigation. New points of view on thyroid function in health and disease, *Arch. Int. Med.*, 1918, xxii, 187.

[8] Swingle, W. W., The acceleration of metamorphosis in frog larvæ by thyroid feeding, and the effects upon the alimentary tract and sex glands, *J. Exp. Zool.*, 1917–18, xxiv, 521.

erating effect upon metamorphosis both in normal and thyroidless frog larvæ might be due to constant irritation of the body epithelium. Moreover, bromine, besides being an irritant to the epithelium, is closely related chemically to iodine, and it has been recently asserted by Kendall[9] that the substitution of bromine for the iodine molecule in his substance thyroxin isolated from the thyroid gland would not alter the gross chemical nature or physiological activity of this so called active thyroid principle. All this being true, interesting results were anticipated when bromine was fed to frog larvæ. As a matter of fact, however, only negative results were obtained, even when very strong concentrations were fed over a long period of time. There was absolutely no observable effect upon growth or differentiation. It seems useless to state in detail the entire experiment, so only a brief summary will be given here. Some account is necessary because of the theoretical importance of such negative findings.

The bromine cultures were in all respects similar to the iodine cultures; method of feeding, amounts fed, and dates of examinations were also the same. It was soon observed that all the larvæ in Cultures 3, 4, and 5 showed pronounced tail-fin atrophy. In several of the animals the delicate tissues were literally eaten away, the action of the bromine thus simulating in a superficial way the action of iodine and thyroid extract. None of the bromine-fed animals developed legs or limb buds any faster than the controls, whereas the tadpoles fed equivalent amounts of iodine underwent marked metamorphic changes. The cause of the pronounced tail atrophy among the larvæ at once became apparent when the tails of the animals were exposed for 20 or 30 seconds over the mouth of the stock bromine solution. The fumes attacked the delicate tissues, literally shriveling them up. An animal treated in this way will generally lose within 3 or 4 days that portion of the tail so treated. This artificially induced atrophy in no way resembles true physiological atrophy and resorption. Iodine in strong concentration will have a slight caustic effect on the tail in early stages of the experiment, though the effect is slight compared with that of bromine.

[9] Kendall, E. C., Thyroid hormone and its relation to the other ductless glands, *Endocrinology*, 1918, ii, 81; Studies of the active constituent in crystalline form of the thyroid, *Tr. Assn. Am. Phys.*, 1916, xxxi, 134.

During the latter part of the experiment three and four times as much bromine (chemically equivalent to the iodine) was fed as was given in the highest concentrated iodine culture, but the only effects produced on the larvæ were atrophy of the tail and slight emaciation. Proper precautionary measures were taken that the bromine did not pass out of solution as fumes, thus weakening the concentration. It may be added that this experiment was tried out on two species of Anura, *Rana sylvatica* and *Rana catesbiana*, with negative results in each case.

Discussion of Bromine Feeding.

This experiment though negative has considerable theoretical interest in its bearing upon the relation of iodine to the thyroid. Kendall,[9] who claims to have isolated and synthesized the physiologically active principle of the thyroid, lays especial emphasis upon the presence of the oxy-indol nucleus of his compound, thyroxin, and considers the presence of iodine in the combination of little importance as it acts probably as a catalyst and in no other way. He says in this regard: "The presence of iodine in the compound must exert some influence, and this influence is probably that of rendering the active groups (CO—NH) more reactive." He thinks that if the iodine were absent from his thyroxin, it would take merely a greater "working pressure" to bring about the reaction of the thyroxin. "The substitution of iodine by hydrogen or chlorine or bromine would undoubtedly be followed by an alteration in the degree of reactivity of the substance, but its gross chemical nature and properties would not be altered thereby. That iodine breaks off from the molecule and is used as iodine *per se* for any purpose seems absolutely impossible."

Dr. Kendall's work deserves careful consideration. However, the writer finds it impossible, in the light of the bromine experiment just recorded, to believe that this halogen can substitute in any way for iodine in its physiological activity. Split off the iodine molecule from the thyroxin complex isolated by Kendall and probably the residue would be a physiologically inert substance.

If the gross chemical nature and properties of thyroxin would not be altered by splitting off the iodine molecule, how is it that thyroidless tadpoles, which never undergo metamorphosis under normal conditions, nevertheless quickly transform into frogs if fed

inorganic iodine? It would seem that if iodine is merely an activator of the thyroxin of the thyroid gland, then every cell in the organism elaborates thyroxin or else a thyroidless tadpole could not metamorphose under the stimulus of inorganic iodine. A view that appeals to the writer is to assume that iodine when taken into the body acts upon all the cells of the organism by stimulating intracellular oxidations in each cell and tissue. It is unnecessary to assume that iodine is used *per se* for any purpose, and from this viewpoint there is nothing against the idea that iodine acts as an organic catalyst.[10]

It has been shown that patients with complete atrophy of the thyroid have basal metabolic rates 40 per cent below normal. The question arises as to what maintains energy output from 100 per cent below normal, which would mean death, up to 40 per cent below normal, the point to which basal metabolism sinks in the absence of the thyroid. Kendall attempts an answer to this question which he himself raises, by assuming that there are other chemical substances in the body possessing the same grouping that occurs in his thyroxin. Such substances according to Kendall are amino-acids and proteins, creatine and creatinine. A more probable assumption, in the light of the iodine experiment on thyroidless tadpoles, is that the basal metabolism of patients suffering from athyreosis is prevented from sinking below 40 per cent by the iodine which is taken into the body in their food and drink, and which is absorbed and used by the cells and tissues of the organism without the necessity of its undergoing transformation in the thyroid gland tissue. This is exactly what occurs when iodine is fed thyroidless tadpoles. Moreover, the basal metabolism of thyroidless frog larvæ is low. Apparently, what keeps it sufficiently high for the continuance of the life processes is the minute quantities of iodine taken into the body in the food and water.

However iodine may act physiologically, the writer feels certain of his ground in asserting that bromine cannot be substituted for it.

[10] The writer suggested in a recent paper[1] that the thyroid gland probably did not elaborate an internal secretion. This meant that the gland did no more than the other cells and tissues of the organism could do, if they were given the appropriate conditions. Certainly the evidence obtained from the metamorphosis of thyroidless tadpoles under the stimulus of iodine feeding strongly indicates this.

Microscopic Examination of the Thyroids.

The glands of the tadpoles were preserved for study of the gross and histological structure after the method previously described.[1] They were first studied whole, then sectioned.

Frog larvæ of equal age, reared under identical environmental conditions, vary considerably in size, hence it is necessary, in order to obtain a true picture of thyroid conditions in the various cultures, to preserve animals as nearly equal in size as possible. The thyroids increase in size as the organism grows, consequently no comparison is possible between thyroids of large and small larvæ, even though taken from the same culture.

Examination of the algæ-fed controls and bromine-fed animals failed to reveal any differences either in size of the thyroids or in the colloid content between the two cultures. Bromine feeding apparently has no effect on the thyroid gland.

On the other hand, however, a comparison of the thyroids of animals taken from Iodine Culture 5 with those from algæ-fed controls of similar size showed little if any difference in the gross size and structure, but a difference in the colloid content of the follicles of the two sets of animals. Colloid is present in the thyroid follicles of the algæ-fed larvæ but is rather scanty in amount and thin in consistency compared to that found in glands from animals fed iodine over long periods.

In an abstract presented recently before the Anatomical Association[11] Allen states that "iodine feeding does not cause any marked increase in colloid deposition in the thyroid glands of pituitaryless tadpoles." This statement, though apparently contradictory to the writer's results, nevertheless is in line with them. In the first place extirpation of the pituitary gland, not only in Anura but in all species investigated, leads apparently to imperfect development of the thyroid, and it is probably because of this malformation and consequently malfunctioning of the thyroid that pituitaryless frog larvæ do not normally metamorphose. Hence such animals will react to iodine feeding just

[11] Allen, B. M., The relation of the pituitary and thyroid glands of *Bufo* and *Rana* to iodin and metamorphosis, *35th Session Am. Assn. Anat., Abstracts*, 1919.

as the writer previously found thyroidless tadpoles did. Tissues and cells, other than those of the thyroid, assimilate and use the iodine taken into the organism. The need for iodine is great, hence the feeding of iodine should result in no marked deposition of colloid in the imperfect thyroids of pituitaryless animals.

In the second place, the iodine mixture used by Professor Allen in his experiment (starch iodide) was obtained from the writer and represented a 1: 100 mixture of iodine and flour. The pituitaryless animals probably had long passed their normal period of metamorphosis and hence required a smaller amount of iodine and flour to enable them to undergo metamorphosis.

The writer feels certain that prolonged iodine feeding increases the colloid content of the thyroid, both in amount and consistency of the colloid, and that the results with pituitaryless animals are due to the reasons stated.

CONCLUSIONS.

1. Amphibian metamorphosis depends upon the amount of iodine secured by the larvæ; the greater the quantity the more rapid the differentiation.

2. Bromine is physiologically inert when fed even in large quantities to frog larvæ, hence it cannot be substituted for iodine. Bromine feeding has no effect on the thyroid.

3. Iodine is the active constituent of the thyroid gland, in the Anura at any rate, and functions within the body by stimulating intracellular oxidations; it is apparently specific in its action.

4. The basal metabolism of patients suffering from athyreosis, whose metabolism is 40 per cent below normal, is very likely held at this figure and prevented from sinking lower to the death point by the introduction of iodine into the body through food and water.

5. The thyroid gland is an organ the function of which is the extraction from the circulation, storage, and supplying to the organism, under the pressure of its needs, the small quantities of iodine taken into the body. The chief function of this gland then is the utilization of iodine in small quantities.

THE EFFECT OF VARIOUS ACIDS ON THE DIGESTION OF PROTEINS BY PEPSIN.

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I.

The widespread occurrence of antagonistic salt action on living tissues raises the question whether the underlying cause of the phenomenon might not be found in the action of the salts on the activity of the enzymes. A similar effect has been described by Falk[1] in the case of lipase. As Loeb[2,3] has shown, it is also possible to demonstrate antagonistic salt action on the physical properties of a protein; *i.e.*, gelatin. It seemed important therefore to determine whether or not such an effect was to be found in the influence of various acids on the digestion of proteins by pepsin.

The relative action of the various acids on the pepsin digestion of proteins has already been the subject of many investigations.[4] The literature on the subject is confused and contradictory, however, due largely to the fact that in the early work the effect of the hydrogen ion concentration was not taken into account and that the acids were usually compared in equimolecular or even equipercentage strengths. Attempts were made by Berg and Gies[5] to rule out this disturbing factor by using solutions containing equal calculated amounts of hydrogen ion. They took no account, however, of the "buffer" action of the weaker acids and of the proteins so that their

[1] Falk, I. S., *J. Biol. Chem.*, 1918, xxxvi, 229.

[2] Loeb, J., *J. Biol. Chem.*, 1918, xxxiii, 531; *J. Gen. Physiol.*, 1918–19, i, 39, 363, 483, 559.

[3] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 237.

[4] Pfleiderer, R., *Arch. ges. Physiol.*, 1897, lxvi, 605. A review of the early literature is given in this article.

[5] Berg, W. N., and Gies, W. J., *J. Biol. Chem.*, 1906–07, ii, 489.

solutions probably did not have the same hydrogen ion concentration. Sörensen[6] has shown that the activity of pepsin depends very largely on the hydrogen ion concentration, and at the same time has developed accurate methods for the determination of the latter. In view of his work it is obvious that the comparison of the action of the different acids must be made in such a way as to keep this factor constant. Failure by the earlier workers to do this probably accounts in large measure for the great differences in the observed efficiency of the various acids.

More recently Michaelis and Mendelssohn[7] have shown that the optimum acidity for the digestion of edestin by pepsin is the same for hydrochloric, nitric, tartaric, and oxalic acids. Ringer[8] states that the optimum reaction depends on the protein used and coincides with the maximum hydration of the protein as measured by the viscosity. A somewhat similar theory was proposed by Pfleiderer[4] who attempted to show a relation between the rate of digestion of fibrin and the amount of swelling in various acids.

In all these investigations the amount of digestion was followed by determining (1) the amount of solution of an insoluble substrate, (2) the amount of precipitable protein left in solution, or (3) the rate of liberation of carmine from carmine fibrin. As has been pointed out by various authors, there is considerable doubt as to whether any of these methods actually follows the chemical changes in the structure of the protein during hydrolysis.

The recent improvements in the technique of the determination of amino nitrogen by the Van Slyke[9] method make it possible to follow the increase in the number of free amino groups. From our knowledge of the changes involved in the hydrolysis of proteins it would seem that this increase probably follows accurately the amount of hydrolysis. The changes are small, however, and even with the greatest care it is difficult to get strictly accurate figures.

[6] Sörensen, S. P. L., *Biochem. Z.*, 1909, xxi, 131.

[7] Michaelis, L., and Mendelssohn, A., *Biochem. Z.*, 1914, lxv, 1.

[8] Ringer, W. E., *Kolloid-Z.*, 1916, xix, 253; *Arch. néerl. de Physiol.*, abstracted in *Physiol. Abstr.*, 1919, iii, 408.

[9] Van Slyke, D. D., *J. Biol. Chem.*, 1913–14, xvi, 121.

In the present work the rate of pepsin digestion of gelatin, egg albumin, edestin, blood albumin, and casein in the presence of hydrochloric, nitric, acetic, sulfuric, oxalic, phosphoric, and citric acids has been followed by this method. The determinations were made at two ranges of hydrogen ion concentration, pH 1.0 to 1.5 and pH 2.5 to 3.5.

A summary of the results obtained with edestin at a reaction of pH 2.6 is given in Table I. The results obtained with the other pro-

TABLE I.

Quantity of Amino Nitrogen per 10 cc. of Solution at 24°C. and 750 mm.

Substrate, 20 cc. of edestin solution A.
Pepsin, 20 cc. of solution A.
} Final volume, 120 cc.

Experiment No.	Time.	Acid.							
		HCl	HNO_3	Acetic	H_2SO_4	Oxalic	H_3PO_4	Citric	HCl*
	hrs.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0	0.50	0.49	0.49	0.48	0.51	0.50	0.49	0.49
2	0	0.50	0.50	0.48	0.49	0.48	0.51	0.48	0.49
1	4	1.28	1.23	1.09	1.33	1.22	1.24	1.20	0.50
2	4	1.25	1.21	1.16	1.25	1.26	1.25	1.24	0.47
1	24	1.55	1.58	1.17	1.56	1.48	1.44	1.39	0.51
2	24	1.51	1.50	1.37	1.48	1.50	1.52	1.49	0.50
		pH of solution.							
1	24	2.6	2.6	2.6	2.5	2.4	2.2	2.2	2.5
2	24	2.5	2.7	2.6	2.4	2.7	2.4	2.3	2.3

* Control with boiled pepsin solution.

teins were practically identical with these and therefore will not be given here. The experiments show that the rate of hydrolysis of all the proteins studied is identical for all the acids (except acetic) within the rather wide range of error of the method used (about 5 per cent). With gelatin acetic acid also behaves quantitatively like the other acids even in concentrations as high as 25 volumes per cent. With the other proteins the rate of hydrolysis in the presence of acetic acid is slower than with HCl, HNO_3, H_2SO_4, oxalic, citric, or phosphoric acids. The effect therefore is evidently on the protein

and not on the enzyme. These experiments also show that the physical properties of the solution, such as viscosity, have little or no effect on the rate of digestion since Loeb has shown that there is a striking difference in the viscosity of gelatin in sulfuric as contrasted with hydrochloric acid solution. This is still more strikingly shown in the experiments with edestin, which is practically insoluble in sulfuric acid and yet digests under these conditions at the same rate as when dissolved in hydrochloric acid. The simplest explanation of these results would seem to be that the rate of digestion of the protein is determined by the amount of acid protein salt formed. As Loeb[2,3] has shown, the physical properties of a gelatin solution are also functions of this same quantity.

II. EXPERIMENTAL.

Method of Keeping Samples.—Samples were withdrawn from the bottles containing the digestion mixtures at intervals of 4 and 24 hours and placed in iced bottles containing three drops of saturated ferric chloride solution. They were kept at 2–4°C. until analyzed. No change could be noted in the amino nitrogen content in 24 hours under these conditions.

Temperature.—Some of the experiments were conducted at 37° ± 0.1, and some at 35° ± 0.1.

Analysis.—The technique was the same as described by Van Slyke except that 10 cc. of solution were analyzed in a large size apparatus and the gas was measured in a small (3 cc.) burette. This was necessary owing to the small amount of amino nitrogen present. The protein solution was allowed to stand 15 minutes in contact with the nitrous acid and then shaken rapidly for 5 minutes. The reaction was complete under these conditions.

Pepsin Solution A.—30 gm. of Fairchilds pepsin were dissolved in 500 cc. of water and allowed to digest at 37° for 24 hours. The solution was dialyzed and filtered, and made up to 3 liters. It contained 0.1 cc. of amino nitrogen per 10 cc. This quantity did not further increase digestion in 24 hours and therefore does not enter into the figures obtained for the increase of amino nitrogen in the solutions.

It was found that 20 cc. of this solution added to 100 cc. of the protein solution caused the hydrolysis to be about one-third complete in 4 hours and two-thirds complete in 24 hours. This concentration was therefore used.

Edestin Solution A.—25 gm. of crystalline edestin were dissolved in 300 cc. of dilute NaOH and precipitated by the addition of dilute HCL. The reaction was adjusted to the isoelectric point of edestin and the solution then dialyzed for a week against tap water and 2 days against distilled water. It was then diluted to 500 cc. A fine suspension was obtained which could be accurately pipetted. The conductivity was about that of an M/1,500 KCL solution, showing that only traces of electrolytes were present. The other protein solutions were purified in the same way by dialysis at the isoelectric point (Loeb).[3]

Adjustment and Measurement of the Reaction.

The required amount of protein solution was pipetted into a 100 cc. volumetric flask and a drop of indicator added (methyl orange or thymol blue, depending on the reaction desired). Hydrochloric acid was then added until the approximate reaction desired was reached. The solutions containing the other acids were prepared in the same way by adding the acid to the protein solution until the color matched exactly that of the flask containing the hydrochloric acid solution. In this way solutions of the same pH could be easily prepared.

Control experiments showed that the indicator had no effect on either the rate of digestion or the analysis. The absolute reaction of the mixtures could not be measured colorimetrically owing to the "protein error." A sample of the solution was removed shortly after adding the pepsin therefore and the pH determined by the E.M.F. method. It was found as stated by Sörensen that the change in reaction during the digestion was insignificant.

SUMMARY.

1. At equal hydrogen ion concentration the rate of pepsin digestion of gelatin, egg albumin, blood albumin, casein, and edestin is the same in solutions of hydrochloric, nitric, sulfuric, oxalic, citric, and phosphoric acids. Acetic acid diminishes the rate of digestion of all the proteins except gelatin.

2. There is no evidence of antagonistic salt action in the effect of acids on the pepsin digestion of proteins.

3. The state of aggregation of the protein, *i.e.* whether in solution or not, and the viscosity of the solution have no marked influence on the rate of digestion of the protein.

The author is indebted to Drs. Van Slyke and Cullen for placing their apparatus for the hydrogen ion determination at his disposal.

THE RESPIRATORY RATE OF THE SCIATIC NERVE OF THE FROG IN REST AND ACTIVITY.

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(Received for publication, May 22, 1919.)

It has been accepted generally that nerve fibers have extremely low metabolic activity and that their oxidation processes in no way increase as the result of excitation. Such conclusions are based on the facts that (1) tetanization does not increase heat production in the nerve,[1] and (2) the nerve cannot be fatigued by stimulation. Furthermore, the well known fact that the nerve trunks are poorly supplied with blood vessels weighs against any assumption of the dependence of physiological function upon a high degree of metabolic activity.

On the other hand, it has been shown by Waller[2] that the intensity of the reponse of nerve fibers to excitation is increased by the application of carbon dioxide, and that nerves show the staircase phenomenon when tetanized. From this the conclusion has been drawn that carbon dioxide is produced by the nerve itself as a result of activity, and that this carbon dioxide acts further to increase the sensitivity of the tissue, thus resulting in the staircase effect. It should be pointed out that there are many substances which increase the sensitivity of nerve fiber, for example, salts like the sulfates, oxalates, or citrates. In fact Waller does not insist on the fact of carbon dioxide production by nerve, but says that the staircase effect may be due to some other substances.[3]

Recently, Tashiro[4] attempted directly to estimate the amount of carbon dioxide given off by the excised nerves of frogs and crabs.

[1] Hill, A. V., *J. Physiol.*, 1911–12, xliii, 433.

[2] Waller, A. D., *Proc. Roy. Soc. London*, 1895–96, lix, 308.

[3] Waller, A. D., Physiology, the servant of medicine, London, 1910, 43.

[4] Tashiro, S., A chemical sign of life, Chicago, 1917.

An elaborate apparatus, to which the inventors have given the name "biometer," was used to collect the carbon dioxide produced by the tissue and to bring it into contact with a drop of barium hydroxide solution. The formation of the precipitate of barium carbonate was observed under the microscope. The time required for the formation of an amount of carbon dioxide sufficient to cover the drop with a film of barium carbonate was used as a measure of the rate of carbon dioxide production. With this method, Tashiro has estimated the frog's sciatic nerve to give off carbon dioxide at the rate of 5.5 $\times 10^{-7}$ gm. per 10 mg. of tissue per 10 minutes. He compares this result with the data obtained by other workers for the rate of carbon dioxide output of the entire animal and of muscle tissue. From such a comparison he concludes that nerves have not only a carbon dioxide metabolism equal to that of other tissues but greater.[5]

With a view to getting at the facts regarding carbon dioxide production in nervous tissue, the writer has employed the indicator method developed by Haas[6] in Osterhout's laboratory and subsequently used by Osterhout and his pupils.[7] In practice a quantity of the indicator, phenolsulfonephthalein, sufficient to give the desired tint was added to Ringer's solution. A liter of this solution in a glass-stoppered bottle was brought to the required reaction by a few drops of 0.1 N NaOH. For the measurements, small tubes of Pyrex glass calibrated to hold 3 cc. of solution were used. These were closed with paraffined corks. Both corks and tubes were boiled repeatedly in neutral water, and before being used were washed in alcohol, then in ether, and finally the corks were saturated with hot neutral paraffin. The tubes were next tested for several hours by filling with the tinted solution, corking, and inverting. Those in which any color change occurred were discarded. This procedure was carried out before each experiment. In the experiments, the rate of carbon dioxide production was determined by measuring the time necessary for the tint to change from pH 7.8 to 7.4. The

[5] Mathews, A. P., Physiological chemistry. A text-book and manual for students, New York, 1915, 590.

[6] Haas, A. R., *Science*, 1916, xliv, 105.

[7] Osterhout, W. J. V., Gustafson, F. G., Brooks, M. M., Thomas, H. S., Irwin, M., *J. Gen. Physiol.*, 1918, i, 171–209.

end-point was determined by matching the experimental tube with another containing a solution having a tint corresponding to pH 7.4. The matching was done by diffuse daylight or by the light of a daylight lamp, and the time taken with a stop-watch. The experimental error is not over 10 per cent. Obviously the method measures only the rate and not the absolute amounts of carbon dioxide produced. But since the metabolism of nervous tissue is of the greatest significance when compared with that of other tissues, and since the problem of stimulation is entirely a question of comparative rates, any attempt to determine absolute values is, for us, beside the point.

Some of the earlier workers[8] in attempting to determine the reaction of nerve substance to indicators had found the cut surface of the gray matter in the brain and cord to be acid to litmus and other indicators. I have shown that the brain, cord, and sciatic nerves of frogs upon being cut or crushed give an acid reaction with phenolsulfonephthalein.[9] If the sciatic nerve of a frog is kept for half an hour in alkaline (pH = 8.0) Ringer's solution saturated with the indicator, the nerve takes up some of the stain and appears pink, except at the cut end where it is yellow; *viz.*, acid. Microscopic examination shows that the cut end of each fiber is yellow. If the stained nerve is crushed on a glass plate with a glass rod, the entire tissue turns yellow. The same result follows if the nerve is allowed to dry in the air or in CO_2-free air, while neutral cotton tinted with the indicator and treated in the same way shows no change in reaction. This acid of injury is not due to stimulation, since it is strictly localized at the points of cutting or crushing.

It can be shown that all the acid given off by nervous tissue and the greater part of that given off by muscle is carbon dioxide. For this purpose the tissue is put into a tube of tinted Ringer's solution previously freed from carbon dioxide by aspirating with CO_2-free air. When a certain amount of acid has been formed, the tissue is removed and the solution again aspirated with CO_2-free air. This treatment removes all the acid formed by nervous tissue, as shown

[8] Pflüger, E., *Arch. ges. Physiol.*, 1875, x, 312.

[9] Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 18.

by the recovery of the tint corresponding to the initial pH value of the solution. By the use of Hopkins' thiophene test I have found that brain tissue produces small amounts of lactic acid as the result of cooking. But any lactic acid produced by injury to nervous tissue in my experiments presumably reacted with the carbonates of the tissue causing carbon dioxide to be given off.

It is apparent that the first problem in determining tissue respiration is to eliminate the acid of injury from the measurements. This proved to be a simpler matter than at first seemed possible, because it was found that the acid is given off only during a very short period after injury. This can be shown by determining the rate of acid

TABLE I.

Effect of Crushing on Rate of Acid Production.

Sciatic nerve.			
Condition.	Reaction time.		Rate.*
		sec.	
Resting.		1,620	0.06
Crushed.	Successive measurements.	420	0.24
		390	0.26
		790	0.13
		1,500	0.07

* Rate $= \frac{100}{\text{seconds}}$. Temperature 18–19°C.

output of a sciatic nerve, before and after crushing. The rate increases at once as a result of the acid produced by the injury but falls rapidly to the original rate. This indicates the momentary character of the production of the acid of injury and proves its rapid elimination.[10] Table I illustrates this point.

It is therefore possible by making a number of successive readings for each point, and by discarding the first readings, to obtain a fairly accurate estimation of the respiratory rate of the tissue by this method. In case the periods of time were not long, *i.e.* less than 3 minutes, five successive readings were taken, and an average of the

[10] Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1918–19, xvi, 35.

last two used as the true value, figures being given to the nearest 5 seconds. When the time extended over 5 minutes it was possible to make only one or two readings, the time being given to the nearest half minute.

The first point to be settled is: Does a nerve trunk give off carbon dioxide at a rate equal to that shown by muscle and by central nervous system tissue? For the purpose of answering this, equal weights of sciatic nerve, sartorius muscle, and brain were taken from the same frog. Measurements of the rate of acid production were made as soon as the tissues were removed from the body and at intervals of an hour subsequently. It was found that if the tissues were kept in a small quantity of Ringer's solution during the time intervening between the measurements the acid production increased again after 2 hours, coincident with the rigor and opacity of the muscle. If, however, the pieces of tissue were kept in a large quantity of neutral Ringer's solution during the time interval, there was a falling off in the rate each hour for about 4 hours. Table II and the corresponding graphs (Figs. 1 and 2) show the relative respiratory rates of the three types of tissue and the decrease in rate with time. A large number of experiments of this character were made, and in all cases it was found that central nervous system tissue and muscle showed a high rate, while sciatic nerves always gave a rate of much lower order; *viz.*, 10 to 30 per cent of that of the other tissues. Just what part of this carbon dioxide is due to the metabolism of the axis cylinder and what to the connective tissue cells of the nerve trunk remains to be determined.

The question of greatest interest in connection with the metabolism of nervous tissue is: Does the functional activity of the nerve cause an increase in the production of carbon dioxide? Tashiro has answered this in the affirmative, and states that stimulation of a nerve more than doubles the rate of carbon dioxide production. With a view to getting additional evidence on this point, I stimulated sciatic nerves with induction shocks while the acid measurements were being made.[11] For this purpose fine copper wires were led into the tube beside the cork. The ends of the wires were bent

[11] Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1918–19, xvi, 66.

hook-shape and the nerves wound around them. The hooks reached to within 1 to 2 mm. of the surface of the solution and the suspended tissue dipped into it. Stimulation was made with a Harvard inductorium, giving maximal shocks. Readings were first made with

TABLE II.*

A, Weight of Each Tissue 140 mg.

	Medulla.		Muscle.		Sciatic.		$\frac{\text{Sciatic}}{\text{Medulla}}$ (Ratio of rates).	$\frac{\text{Sciatic}}{\text{Muscle}}$ (Ratio of rates).
	Time.	Rate.	Time.	Rate.	Time.	Rate.		
hr.	*sec.*		*sec.*		*sec.*			
0	30	3.3	70	1.43	270	0.37	0.11	0.26
1	60	1.7	90	1.11	540	0.185	0.11	0.17
2	100	1.0	120	0.83	1,000	0.10	0.10	0.12
3	120	0.83	190	0.52	1,200	0.07	0.08	0.13
4	145	0.69	220	0.45	1,200	0.07	0.10	0.15
Average							0.10	0.17

B, Weight of Each Tissue 50 mg.

	Optic lobes.		Muscle.		Sciatic.		$\frac{\text{Sciatic}}{\text{Optic lobes}}$ (Ratio of rates).	$\frac{\text{Sciatic}}{\text{Muscle}}$ (Ratio of rates).
	Time.	Rate.	Time.	Rate.	Time.	Rate.		
hr.	*sec.*		*sec.*		*sec.*			
0.0	50	2.0	60	1.7	240	0.41	0.20	0.24
0.5	65	1.54	55	1.8	300	0.33	0.21	0.18
1.0	130	0.77	120	0.83	600	0.17	0.22	0.20
2.5	190	0.52	225	0.44	1,020	0.10	0.19	0.23
3.5	220	0.45	270	0.37	1,440	0.07	0.16	0.19
Average							0.196	0.208

* In the first column are given the intervals of time between the measurements, by hours. The first column under each tissue shows the reaction time in seconds at the end of each time interval. The second column contains the corresponding rates of reaction. Rate $= \frac{100}{\text{seconds}}$. Temperature 18–19°C.

the nerve at rest, then during stimulation, and lastly at rest again. The frequent inversion of the tube kept the tissues bathed with the solution and cooled. With this method it was found impossible constantly or noticeably to increase the rate of carbon dioxide out-

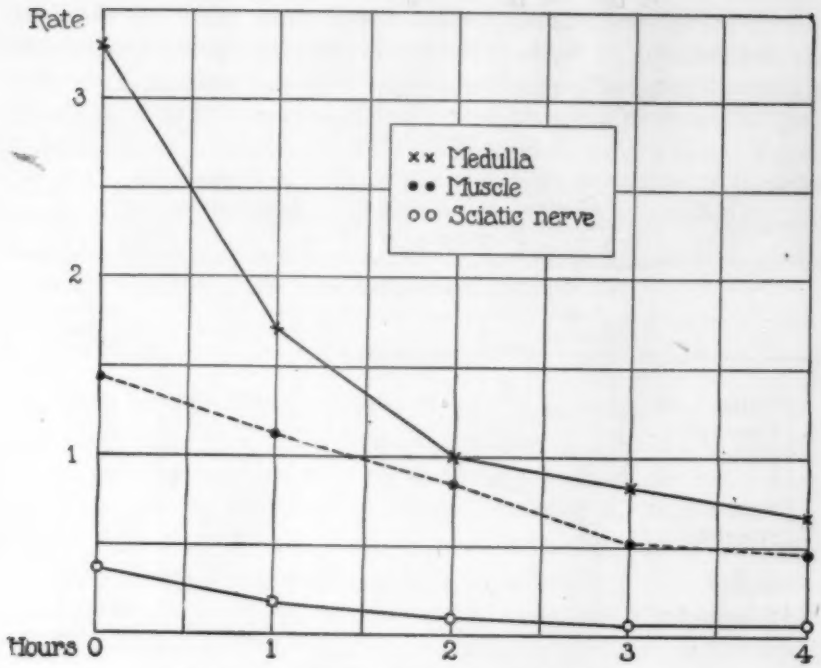

FIG. 1. Constructed from data of Table II A.

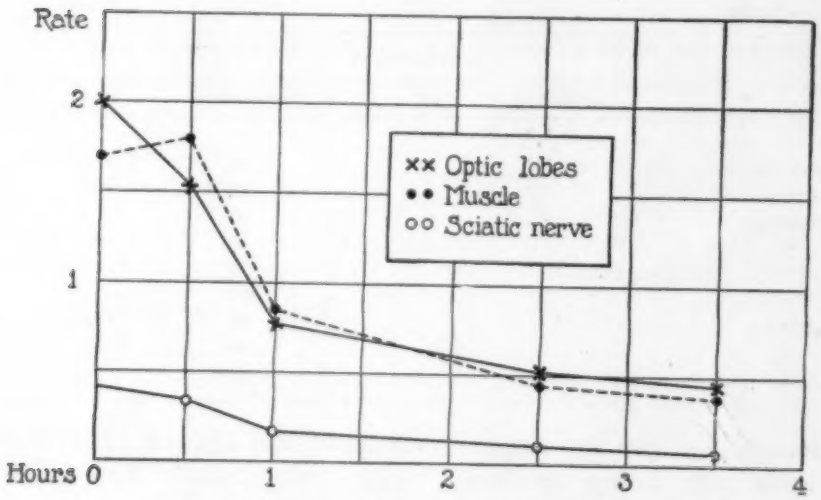

FIG. 2. Constructed from data of Table II B.

put by stimulation, although the nerve in some cases was tetanized for 30 minutes. In Table III are shown the figures for four experiments. A series of controls was made with sartorius muscle. The

TABLE III.

Effects of Stimulation on the Rate of Carbon Dioxide Production in Nerve and Muscle. Individual Animal Denoted by Letters on the Left. Temperature 17–19°C.

Sciatic nerves.

	Condition.	Reaction time.	Rate.
		sec.	
A	Resting	480	0.21
	Stimulated	600	0.17
	Resting	810	0.12
B	Resting	1,800	0.055
	Stimulated	2,100	0.048
C	Resting	1,020	0.098
	Stimulated	930	0.107
	Resting	1,080	0.092
D	Resting	600	0.17
	Stimulated	1,500	0.067
	Resting	1,620	0.062

Sartorius muscle.

Condition.	Reaction time.	Rate.
	sec.	
Resting	85	1.2
Stimulated	95	1.05
Resting	**60**	**1.7**
"	80	1.25
"	95	1.05
Stimulated	100	1.0
Resting	**80**	**1.25**
"	**70**	**1.4**
"	95	1.05

muscle was put on the electrode hooks and allowed to contract isotonically during stimulation. Measurements made during contraction showed no increase, and usually a slight decrease in respira-

tory rate, since the surface was reduced by contraction. Immediately upon relaxation the rate increased from 30 to 100 per cent of that of the resting muscle (see figures under "sartorius muscle" Table III). The method used is therefore adequate to show changes in the metabolic activity of muscle resulting from contraction. Since the same method fails to reveal changes in carbon dioxide production in the nerve as the result of stimulation, it is necessary to conclude that the functional activity of nerve fiber does not depend upon processes resulting in the production of carbon dioxide.

SUMMARY.

1. With the indicator method of Haas, the rates of carbon dioxide production have been measured in the case of the sciatic nerve, various parts of the brain, and the sartorius muscle of the frog. The rate of respiration of the sciatic nerve is from 10 to 30 per cent of that of the other tissues, varying somewhat with the individual.

2. Stimulation of the sciatic nerve with induction shocks sufficient to induce tetanus of the muscle does not increase the output of carbon dioxide from the sciatic nerve, even if continued as long as 30 minutes. Sartorius muscle used as a control showed a marked increase in carbon dioxide production upon relaxation after contraction resulting from such stimulation.

3. These facts indicate that the nerve impulse does not depend upon processes leading to the production of carbon dioxide.

THE GROWTH OF HIGHER PLANTS IN SOILS FREE OF MICROORGANISMS.*

By E. B. FRED.

(From the Laboratory of Agricultural Bacteriology of the University of Wisconsin, Madison.)

(Received for publication, March 20, 1919.)

In a study of the action of bacteria in soil it is frequently desirable to determine the relation that exists between the microorganisms and higher plants. The procedure is comparatively simple if small plants or seedlings are used. For this purpose test-tubes, especially the large Pyrex tubes described by Knudson,[1] give excellent results. Where it is necessary to grow large plants and to carry these to maturity, the procedure is not so simple. On account of the size of the apparatus and the length of time required for the development of large plants, it is difficult to prevent contamination. Some of the points which deserve special care are: (1) sterilization of the apparatus, of the medium, and of the seed; (2) planting of the bacteria-free seed or seedling; (3) adding the sterilized water required for plant growth.

The nature of the medium used for the plants determines the time of sterilization. If a synthetic medium, liquid or solid, is used it rarely requires a long period for sterilization. Soil, however, is extremely difficult to sterilize and in order to kill all the bacterial spores that may be present it is necessary to heat it for a long time and at a high temperature.

The destruction of bacteria on the seed-coats is in the majority of cases readily effected by means of mercuric chloride solution in a partial vacuum. Although a great number of methods have been recommended for the sterilization of seed, I have uniformly secured

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[1] Knudson, L., *Cornell Univ. Agric. Exp. Station, Mem. 9*, 1916, 17.

the best results from the use of mercuric chloride. After the vacuum apparatus is once assembled, the method is easy to manipulate and gives a high percentage of viable seeds free of bacteria. The original design of this apparatus as given by Hutchinson and Miller[2] has been modified. Fig. 1 shows the apparatus ready for use. Heavy walled bottles (1 pint milk) and heavy steam-proof rubber tubing are very convenient.

The preparation of the apparatus for seed sterilization is a simple matter. Bottle A serves to prevent air contamination. The large tube which enters A is packed with non-absorbent cotton and the

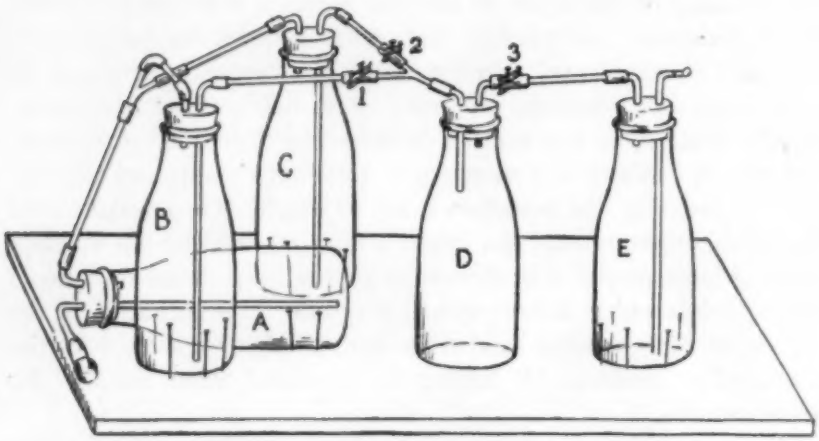


FIG. 1. An apparatus for the sterilization of seeds.

exit tube of D with glass-wool; in the latter the plug prevents the seed from escaping into E. The distilled water and the 0.25 per cent mercuric chloride solution are warmed. When the solutions are about 70–80°C., the mercuric chloride is poured into B, the water into C. The warm solutions prevent the bottles from cracking when sterilized. All rubber stoppers are then fitted tightly into the bottles and the clamps, except 3, are closed.

The entire apparatus is sterilized in the autoclave for 30 minutes at 15 pounds pressure. When cooled to 40°C. the exit tube of E is

[2] Hutchinson, H. B., and Miller, N. H. J., *J. Agric. Sc.*, 1908, iii, 185.

connected to a water pump, preferably with a suction flask between E and the pump.

The seeds are placed in D and the suction pump is started. Clamp 1 is opened and the mercuric chloride allowed to run into D until there is enough of the solution to cover the seeds, then Clamp 1 is closed. The reduced pressure removes the air bubbles from the seed-coats and thus permits the mercuric chloride solution to come in closer contact with the surface of the seed. The time required to destroy the bacteria, as well as spores, depends upon many factors: nature of seed-coat, kind of bacteria, etc. Field peas sterilized for 3 minutes in a 0.25 per cent mercuric chloride solution were found to be entirely free of bacteria. Smaller seeds, as clover and alfalfa, require only from 1 to 2 minutes to be sterilized.

To remove the mercuric chloride, the bottle containing the seeds is inverted. When the liquid is removed, bottle D is returned to its original position and water from C allowed to run in until the seeds are covered. The seeds are then washed several times with the sterilized water from C to remove the mercuric chloride. The amount of water used should be regulated so that C will be empty after the final washing. When the water is removed from D, clamp 3 is closed and clamp 2 is opened slowly in order to restore atmospheric pressure in D. Bottle D is removed and the seeds are transferred directly to a sterile Petri dish.

The importance of growing plants in the absence of bacteria is well recognized and many different types of apparatus have been designed for this purpose.

In 1898 Lauck[3] described a method for the growth of plants without bacteria, but unfortunately the apparatus he used is very complicated, expensive, and difficult to handle. It is recommended for the growth of large sized plants. Schulze[4] in 1901 devised a vessel for growing large plants free of bacteria which has many advantages. The methods described by Petri[5] and by Schmidt[6] were designed especially for young seedlings and, therefore, are not included in this

[3] Lauck, H., *Centr. Bakteriol., 2te Abt.*, 1898, iv, 706.
[4] Schulze, C., *Landw. Jahrb.*, 1901, xxx, 327.
[5] Petri, L., *Centr. Bakteriol., 2te Abt.*, 1909, xxiv, 146.
[6] Schmidt, E. W., *Centr. Bakteriol., 2te Abt.*, 1910, xxv, 426.

brief discussion. Von Beke[7] in 1912 described a vessel for cultivating plants free from microorganisms. In common with many others, his method is also complicated. The same criticism may be offered in regard to the apparatus used by Russell and Hutchinson[8] and by Schulow.[9] The design by Schulow is most ingenious and offers many advantages.

A modification of the Russell and Hutchinson and the Schulow apparatus has been used and found to be fairly satisfactory. The great objection to it is the difficulty of sterilization. The apparatus described in this article is a modification of the one given by Kellerman[10] and has the advantage of being simple and easy to sterilize. All that is required is a metal pot, a large Pyrex glass cylinder, and a large beaker. No stoppers or connecting tubes are used.

The metal pot or can of galvanized iron is made with a neck on the side and a shoulder at the top to support the glass cylinder. This neck serves as an opening through which to plant, to water, and also to aerate. The size of the pot is determined by the kind of plant to be grown. The vessel used had the following dimensions: 18.5 cm. tall, measured outside, and 20 cm. tall if shoulder is included, 11 cm. in diameter at the base and 9 cm. in diameter at the top, due to the shoulder. This shoulder is about 2.5 cm. high outside and 4 cm. inside. The pot holds about 1,700 gm. of air-dry soil. At the bottom of the shoulder on the pot there are two or three small holes which serve to carry off any water which may condense and run down the sides of the glass cylinder. The neck on the side of the pot is just below the shoulder and is about 2 cm. in diameter and about 2.5 cm. long. The Pyrex cylinder is about 9.5 cm. in diameter and about 46 cm. long. The cover consists of a beaker of about 11.5 to 12 cm. in diameter. These three parts constitute the entire apparatus and involve a cost of about three dollars.

The apparatus is simple, easy to sterilize, and can be handled without special care. If soil is used it is sterilized several times in the metal pot. At the final sterilization of the soil, the glass

[7] von Beke, L., *Centr. Bakteriol., 2te Abt.*, 1912, xxxiii, 442.

[8] Russell, E. J., and Hutchinson, H. B., *J. Agric. Sc.*, 1909, iii, 111.

[9] Schulow, I., *Ber. bot. Ges.*, 1911, xxix, 504.

[10] Kellerman, K. F., *U. S. Dept. Agric., Bureau Plant Industry, Bull. 120*, 1914.

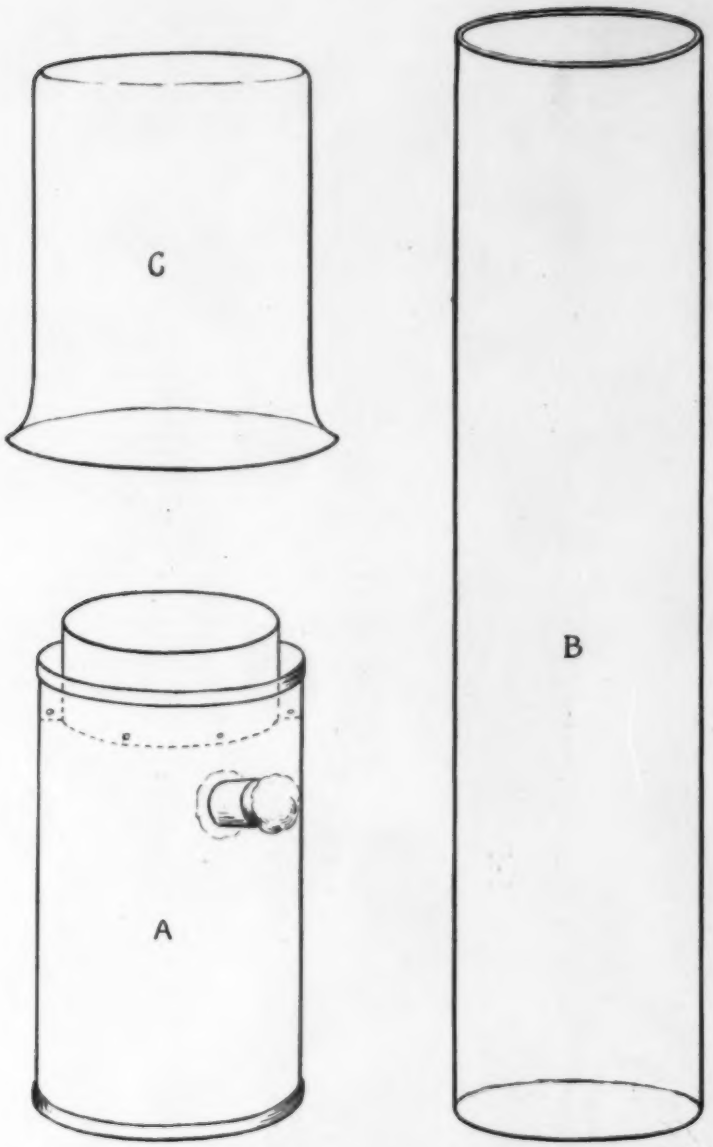


FIG. 2. The parts of the apparatus for growing plants under sterile conditions. A, metal pot; B, Pyrex cylinder; C, beaker.

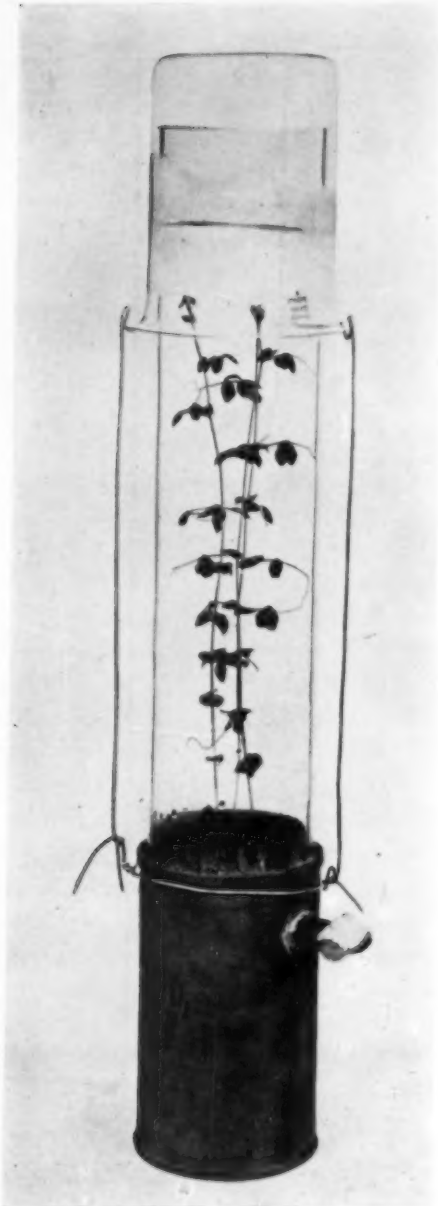


FIG. 3. Showing field peas in sterilized soil. About $\frac{1}{5}$ natural size.

beaker and cylinder are included. The neck is plugged with cotton and the top of the pot is covered with several layers of cotton. One end of the glass cylinder is protected in the same way while the other end is covered with a glass beaker. It is well to have a layer of non-absorbent cotton between the beaker and the glass cylinder. After sterilization, the cotton covers are removed and one end of the cylinder is fitted to the shoulder at the top of the pot. By means of large sterilized pipettes, it is easy to add water through the neck into the pot. The different parts of the apparatus are shown in Fig. 2. In order to give better aeration, the beaker may be raised from the top of the cylinder, as shown in Fig. 3, and wire supports used to hold the beaker in place. This apparatus is easy to handle and may be sterilized repeatedly without cracking.

Several tests have been made of this apparatus under greenhouse conditions. If the apparatus is sterilized and sufficient care is exercised in watering, plants may be grown for several months without danger of becoming contaminated.

THE ETCHING OF MARBLE BY ROOTS IN THE PRESENCE AND ABSENCE OF BACTERIA.*

By E. B. FRED and A. R. C. HAAS.

(*From the Department of Agricultural Bacteriology, University of Wisconsin, Madison.*)

(Received for publication, April 22, 1919.)

In studies on plant nutrition[1] it is essential to investigate the reciprocal effects of the plant and of the soil bacteria upon one another. In a recent paper[2] the writers have shown that germinating legume seeds do not excrete substances in quantities sufficient to exert a toxic action upon the growth of the nodule bacteria of such plants. The present report makes it evident that soil bacteria may play an important part in the nutrition of plants.

It is a well known fact that roots in the presence of bacteria have the power to carry on active solution processes and thereby increase the available plant food in the soil. The means by which such a dissolving action of minerals takes place is a matter of considerable importance.[3] Unfortunately the problem has been complicated[4] by the failure of many investigators[5] to distinguish clearly[6] the factors[7] that are involved. Some interesting observations as to the causes for soil acidity have recently been made.[8,9]

That the acid excretion from roots has the power to etch marble has long been known. This power of roots to dissolve marble be-

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[1] Lyon, T. L., *J. Am. Soc. Agron.*, 1918, x, 313.

[2] Haas, A. R. C., and Fred, E. B., *Soil Science*, (in press).

[3] Haas, A. R., *Proc. Nat. Acad. Sc.*, 1916, ii, 561.

[4] Crocker, W., *Bot. Gaz.*, 1917, lxiii, 422.

[5] Czapek, F., Biochemie der Pflanzen, Jena, 1905, ii, 869.

[6] Kappen, H., *Landw. Vers. Sta.*, 1918, xci, 1.

[7] Johnson, H. V., *Am. J. Bot.*, 1915, ii, 250.

[8] Hoagland, D. R., *Science*, 1918, xlviii, 422.

[9] Gillespie, L. J., and Wise, L. E., *J. Am. Chem. Soc.*, 1918, xl, 796.

comes of greater significance when we consider the observations of Hoffman[10] upon soil productivity. In twenty-seven out of thirty-two cases examined, it was noted that a greater number of bacteria were to be found adjacent to, rather than away from, the roots. The question therefore arises: Are the bacteria to any considerable extent responsible for the degree to which etching takes place? The results of controlled experiments have shown that roots, when growing in soil containing bacteria, possess a greater etching power upon marble than when growing in the complete absence of bacteria. General experiments were made using the apparatus described by Fred[11] for growing higher plants free of bacteria, as well as large Pyrex glass tubes.

Slabs of marble 4.2 cm. wide, 15 cm. long, and 2 cm. thick, were polished by rubbing the marble on cloth soaked with an aqueous solution of oxalic acid, and quickly removing any adhering acid by means of running water. It was arranged to have the polished surface of the marble slabs extend diagonally down the lower part of the culture vessel.

The same weight (250 gm.) of Miami silt loam soil from Madison was placed in each glass tube after the original soil sample was thoroughly mixed. The soil was shaken somewhat, in order to fill all the space about the marble. The tubes were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 2 hours on 3 successive days. The tubes were tested as to their sterility by observing whether or not growth took place after several days, when some of the soil was placed in beef peptone broth. Water was then added to each tube by means of a pipette, until the water content of the soil was 20 per cent by weight.

Canada field peas, that had been sterilized[11] with a 0.25 per cent mercuric chloride solution with reduced pressure, were placed in Petri dishes and covered with Ashby's agar. When the agar had solidified, the plates were inverted and incubated at 27°C. for 2 days. At the end of this period the seeds had germinated and short radicles were projecting through the seed-coats. This treatment

[10] Hoffman, C., *Kansas Univ. Sc. Bull.*, 1914, ix, 81.
[11] Fred, E. B., *J. Gen. Physiol.*, 1918–19, i, 623.

made it possible to make a selection of the germinating seeds and to avoid the use of those which showed contamination. It should be pointed out in this connection that the uninoculated tubes were tested again at the close of the experiments for the presence of micro-organisms.

Two bacteria-free pea seedlings were transferred into each vessel. The seeds were placed directly over the slanted marble slabs. This made it possible for the roots, as they grew downward, to come into intimate contact with the polished surface of the marble. The seeds were covered over with soil. Preliminary experiments showed that the roots of pea seeds, unless covered over with soil, have a tendency to grow along the surface and are tardy in penetrating the soil. This condition may be due in part to the effects of the sterilization upon the soil texture.

The tubes were placed in the greenhouse and were kept at about 60–70° F. When the seeds in the tubes had grown for 7 days, several of the tubes were inoculated. In the first experiments one-half the tubes were inoculated with a water suspension from field soil. Because of the great number of bacteria as well as the different types added in this inoculum, it is not surprising that the plants with bacteria far exceeded in growth the plants without bacteria. Perhaps the nitrifying bacteria caused this increased plant growth. To avoid the action of certain organisms, *e.g.* nitrifying bacteria, nodule bacteria, and molds, another experiment was carried out in which the soil was inoculated with a mixture of pure cultures of bacteria instead of soil extract. The nitrifying bacteria, therefore, were purposely omitted from this mixture. The following organisms were used: *Azotobacter*, *Bacillus aerogenes*, *Bacillus coli*, *Bacillus communior "C,"* *Bacillus communis "B,"* *Bacillus hartlebii*, *Bacillus avaniensis*, *Bacillus mesentericus*, *Bacillus fluorescens liquefaciens*, *Bacillus fluorescens non-liquefaciens*, *Bacillus pyocyaneus*, *Bacillus proteus*, *Bacillus prodigiosus*, *Bacillus tumescens*, *Bacillus subtilis*, *Cladothrix dichotoma*, and *Cladothrix oderifera*.

5 cc. of sterile water were added to each of the uninoculated tubes in order to keep the moisture content of the soils similar in each tube. 25 days after the seeds had been placed in the tubes, the tubes were opened and the slabs and plants examined. A photo-

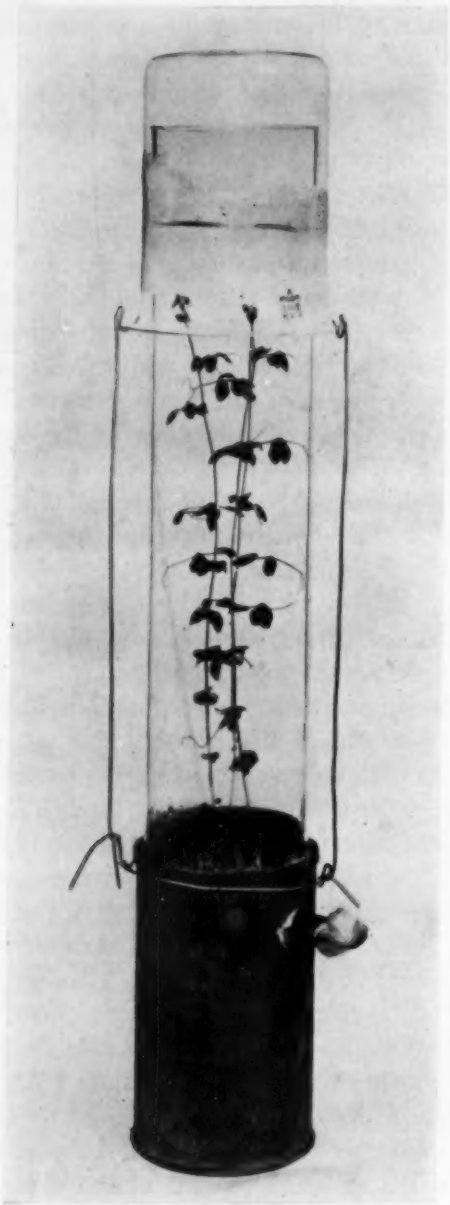


FIG. 1. Photograph of pea plant growing in soil with marble slab present.

graph of one of the pea plants after a period of 42 days is shown in Fig. 1.

Immediately upon opening the uninoculated tubes, samples of the soil were inoculated in tubes of clear nutrient broth. After incubating for several days, the broth tubes were examined for the growth of microorganisms. Whenever any growth was observed, the contaminated tubes were included with the inoculated ones.

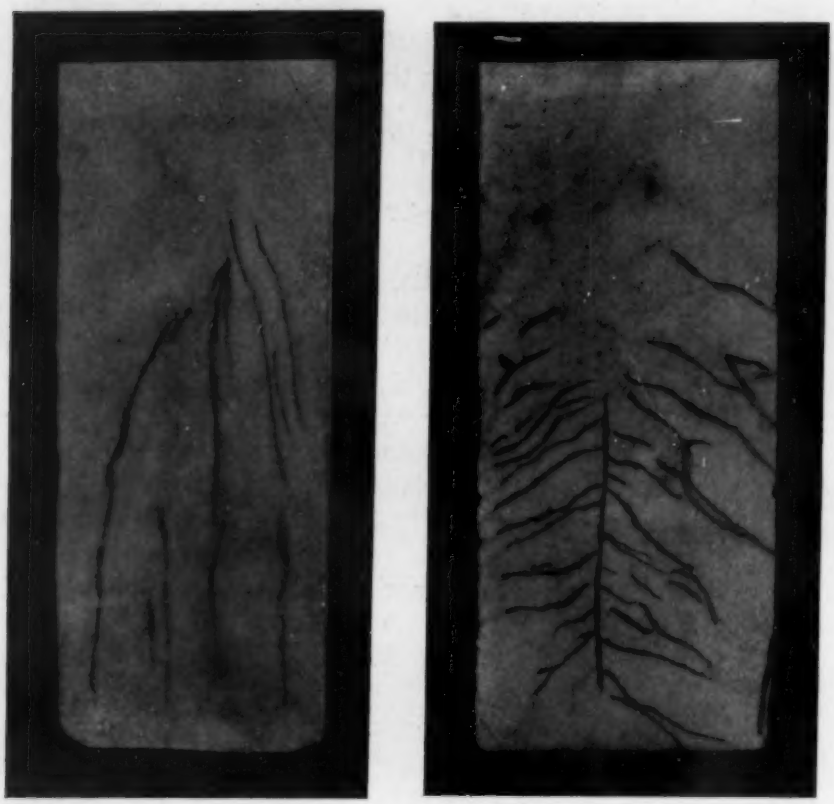
The plants were removed from the tubes by a gentle stream of water. It was found that in nearly every instance the plants in the inoculated tubes made a slightly better growth as regards the length of roots and tops than the plants grown under sterile conditions. An effort was also made to grow plants in agar, but it was found unsatisfactory.

In the preliminary experiments, water-proof drawing ink was rubbed with a smooth cloth into the etchings to bring out the details better in the photograph. Fig. 2, A, shows the effect of the roots upon the marble in the absence of bacteria; Fig. 2, B, shows the effect when the bacteria are present. In the later experiments the etchings were better defined for photographic purposes by carefully filling the etchings with lamp black. Fig. 3, A, shows the effect when the bacteria were absent; Fig. 3, B, shows the effect when the bacteria were present.

The slabs were placed in a row with those from the uninoculated scattered among those from the inoculated tubes. A person unacquainted with the numbers of the slabs was then called upon to arrange the slabs in the order of their etching. In choosing a criterion of etching, the emphasis was put upon the depth to which the etching had taken place. The amount of etching upon the entire polished surface of the slabs was also taken into consideration although here the possibility of some roots not coming into close contact with the marble becomes a factor.

It was found that in every case the marble slabs that had the greatest degree of etching were those taken from the tubes that had been inoculated. It was also found that in many cases it was possible to predict from the order of the slabs whether or not an uninoculated tube had become contaminated before the conclusion of an experiment, for in this event such slabs would usually be classed among the pieces of marble from the inoculated tubes.

It seems probable that the greater etching power of the roots of pea plants in the presence of soil bacteria can be attributed to the normal carbon dioxide excretion of the living root cells, together with carbonic and other acids set free from dead or dying root cells whose decomposition is accelerated by the presence of the bacteria. While



A. B.

Fig. 2. A, plant roots without bacteria; B, plant roots with bacteria.

the results indicate that the dissolving power of roots is in part due to the action of the bacteria, it must be admitted that there are many factors involved. For example, the food supply of a plant must be different in the presence of bacteria from that of a plant in soil free of bacteria.

A. B.

FIG. 3. A, plant roots without bacteria; B, plant roots with bacteria.

SUMMARY.

In a study of the effect of soil bacteria upon the etching power of the roots of Canada field peas upon polished marble, it has been shown that the presence of the bacteria increases the etching power of the roots.

THE INHERITANCE OF A FLUCTUATING CHARACTER.

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(Received for publication, May 23, 1919.)

In the fall of 1909 Morgan observed that the trident-pattern on the thorax of flies of some of the wild stocks of *Drosophila melanogaster* varied both in the intensity of the pigmentation and in the extent to which the pattern was spread over the thorax. The degree of pigmentation and the extent of the pattern seemed to vary together. A scale of grades was devised (Fig. 1) by means of which it was possible to describe rather accurately the different populations dealt with. Thus, Table I and Curve *a* give the distribution of individuals in "Falmouth" wild stock (October, 1911).

Morgan selected throughout the fall and winter of 1909–10 for increased trident-pattern, with no certain progress until January, 1910, when a few flies were found that were considerably darker than any hitherto observed. By breeding from these darker flies a race was quickly obtained which showed no individuals as low in grade as those in Classes I and II and very few in Class III. This stock was called "with" and mass selection in it was continued for about a year (thirty generations) with no apparent result further than possibly to decrease the numbers in the lower grades. It seems clear that the various grades observed in the stock prior to January, 1910 were merely the normal fluctuations of the wild type pattern, and that a definite mutation had then occurred which gave rise to higher grades and a characteristic new distribution. A census taken of this new "with" mutant population (October, 1911) revealed the condition shown in Table I, and by Curve *b*, Fig. 2. The most numerous class in the "with" stock was VII, which was not represented at all among the wild flies.

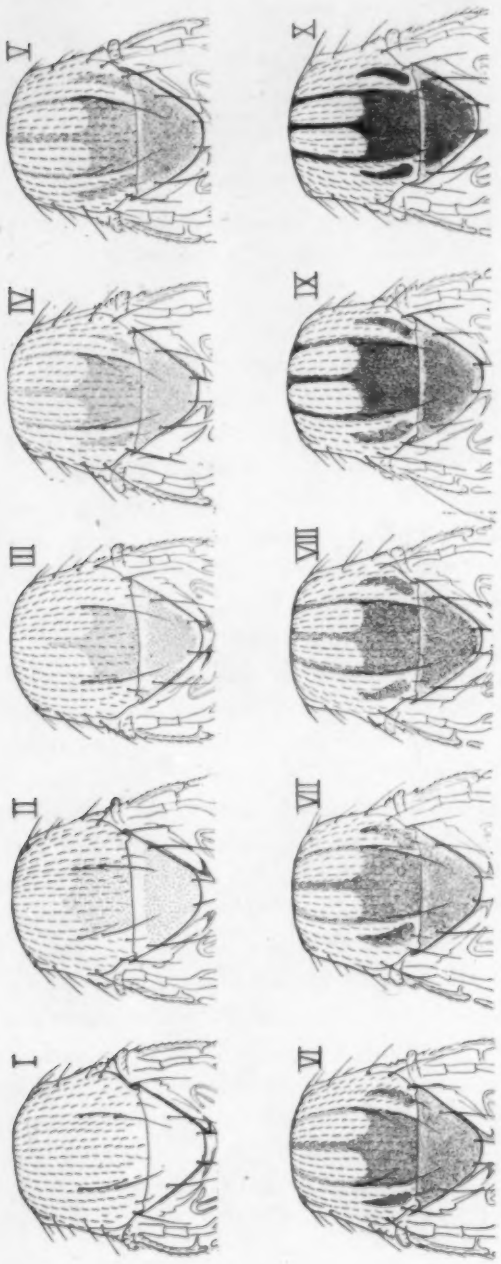

FIG. 1. The scale of grades of the trident-pattern "with."

TABLE I.

Grades of the Trident-Pattern "With" in Various Stocks and Experiments.

Stock or experiment.		"Falmouth" wild stock.		"With" stock.		F_1 "Falmouth" × "with."		F_2 "Falmouth" × "with."		F_2 expectation.	"Super-with" stock.	
		No. of flies.	Per cent.	No. of flies.	Per cent.	No. of flies.	Per cent.	No. of flies.	Per cent.	Per cent.	No. of flies.	Per cent.
Grades of "with" trident-pattern.	I	180	11.1	—	—	32	1.2	118	3.8	3.4	—	—
	II	585	36.3	—	—	393	15.2	621	20.0	16.7	—	—
	III	442	27.4	—	—	697	26.9	773	24.9	20.3	—	—
	IV	275	17.0	365	13.3	700	27.1	578	18.7	21.1	—	—
	V	106	6.6	398	21.8	519	20.1	432	14.0	17.2	—	—
	VI	26	1.6	729	26.6	218	8.4	313	10.1	11.2	—	—
	VII	—	—	773	28.3	28	1.1	233	7.5	7.6	53	18.1
	VIII	—	—	273	10.0	—	—	32	1.0	2.5	65	22.3
	IX	—	—	—	—	—	—	—	—	—	70	24.0
	X	—	—	—	—	—	—	—	—	—	104	35.6
Total.....		1,614		2,538		2,587		3,100			292	

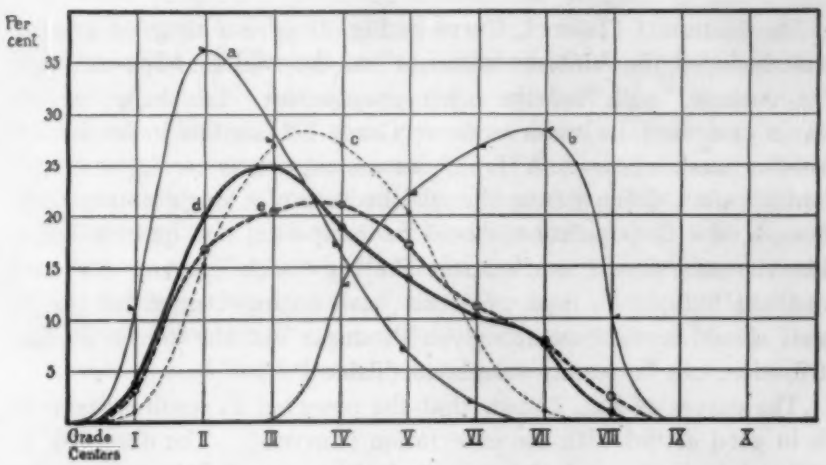

FIG. 2. Curves representing the distribution of grades of the trident-pattern "with" in various stocks and experiments. Curve a = "Falmouth" wild stock, b = "with" stock, c = F_1 from "Falmouth" × "with," d = F_2 from "Falmouth" × "with," e = expected F_2 as calculated from the observed distributions in the parental stocks and F_1. The curves were drawn free-hand to conform to the plotted points.

The F_1 Result of the Cross between "With" and the Wild Stock.

The above classifications were made preparatory to a cross between these two lines. The F_1 flies from the cross of "without" wild flies to "with" flies of the mutant stock were in the main intermediate in grade between the two parental types (Table I, and Curve *c*, Fig. 2). The mode of the F_1 was at Grade IV. There were very few flies that did not show a faint pattern (Grade I = 1.2 per cent). At the other extreme there were no flies that were as dark as Grade VIII and only a very few (1.1 per cent) as dark as Grade VII. A comparison of the F_1 distribution with that of each parent (Fig. 2) shows that, as judged by the means, modes, and extent of overlap, the F_1 population resembles the wild parent more closely than the "with" parent. "With" is classified as a "partial" dominant since it is known to be the mutant type and the F_1 flies show considerable effect of the "with" gene.

The F_2 from the Cross of "With" to Wild.

The F_2 results (Table I, Curve *d*, Fig. 2) gave a range of grades that included the faintest "withouts" of the wild grandparent and the darkest "with" of the other grandparent. In shape the F_2 curve presented its main mode at Grade III and an indication of another mode at Grade VII. If our assumption is correct that the "with" stock differed from the wild because of a single mutant difference, the F_2 population should be composite, one quarter being like the wild parent, one quarter like the "with" parent, and two quarters like the F_1 type. On this basis an expectation for the F_2 that should result from the given P_1 stocks and the known F_1 distribution, can be readily calculated (Table I).

The curves of Fig. 2 show that the observed F_2 result (Curve *d*) is in good accord with the expectation (Curve *e*). The observed F_2 differed from that calculated in that there was slightly too great a proportion in Grade VII (which corresponds to the mode of the "with" stock) and also a similar excess in Grades II and III (which corresponds to the bulk of the wild stock).

It should be noted that had "contamination of genes" been acting, the F_2 should have deviated from the calculated F_2 in the direction

of the F_1 result, so that the curve of the observed F_2 should have lain between the calculated F_2 curve and the F_1 curve. Instead of this the actual deviations were mainly in the opposite direction.

Chromosome Carrying "With."

That the gene for "with" is situated in the third chromosome was determined by means of the F_2 of the cross of pink by "with," which produced no pinks that were surely "with" (higher grades) or, conversely, no sure "withs" that were pink. The higher grades of "with" are the homozygates and the ratio was thus comparable with the 2:1:1:0 ratio ordinarily observed in "repulsion," crosses between two recessives whose genes are carried by the same chromosome.

"Super-With."

The "with" stock used in the preceding experiments had just undergone somewhat over a year of mass selection with little or no observable progress. However, after a few months of selections, decided progress had been obtained in a sister line. A certain culture (E, November, 1910) was seen to contain individuals of still darker grades (IX and X) and selection soon established a "super-with" population. This E line then proved as immovable as before, although selection was practiced continually. At the end of twenty-three more generations a census of this line (September, 1911) showed the condition in Table I. This stock was lost before tests were carried out, but we can judge from its sudden manner of origin and subsequent stability that a second mutation had occurred. Whether this second mutation ("super-with") was a modifier of "with" or an allelomorph of "with" was not determined.

VERMILION-DEFICIENCY.

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(From the Zoölogical Laboratory of Columbia University, New York, and the Carnegie Institution of Washington.)

(Received for publication, April 18, 1919.)

CONTENTS.

Summary 645
Origin and lethal action of vermilion-deficiency 646
Linkage tests of the extent of the deficient region and the disturbance in crossing over in adjacent regions 650
Relation between vermilion-deficiency and vermilion-sable duplication 654
Haploid and cytological tests of the extent of the deficient region 655
Dominant action of deficiencies 655

SUMMARY.

In May, 1916, a culture *of Drosophila melanogaster* showed that a new sex-linked lethal had arisen. The linkage relations indicated that the position of the lethal was in the neighborhood of the sex-linked recessive "vermilion," whose locus in the X chromosome is at 33.0. When females heterozygous for the lethal were outcrossed to vermilion males, all the daughters that received the lethal-bearing chromosome showed vermilion eye-color, though, from the pedigree, vermilion was known to be absent from the ancestry of the mother. The lethal action and the unexpected appearance of vermilion both suggested that this was another instance of the phenomenon called "deficiency;"[1] that is, the loss or "inactivation" of the genes of a section of the X chromosome. The lethal action would then be due to the deficient region including one or more genes necessary for the life of the individual. The appearance of vermilion in females carrying only one vermilion gene would be explainable on the ground that the deficient-bearing

[1] Bridges, C. B., *Genetics*, 1917, ii, 445.

females are virtually haploid for the region including the vermilion locus. Linkage tests showed that the amount of crossing over in the neighborhood of the deficiency was cut down by about five units. Part of this may be attributed to the actual length of the "deficient" region, within which it is probable that no crossing over occurs, and part (probably most) to an alteration in the synaptic relations in the regions immediately adjacent. In more remote regions there was no disturbance or perhaps a slight rise in the frequency of crossing over. Both the local fall and the possible rise in more distant regions would seem to argue that a "pucker" at synapsis had been caused by an actual shortening of the deficient chromosome. That the deficient region extends to the left of the locus of vermilion was indicated by a test in which it was observed that the presence of an extra piece of chromosome including the loci for vermilion and sable ("vermilion-sable duplication") did not neutralize the lethal action of the deficiency. Haploid tests with the other recessive mutations in the neighborhood of vermilion showed that the deficiency was not extensive enough to include their loci. Cytological preparations were made but were unsatisfactory. The stock was finally lost, apparently as the result of injurious action upon viability, fertility, and productivity by the deficiency.

References in this paper are made to the following recessive sex-linked mutations: "facet" eye (*fa*, locus 2.4), "cut" wing-shape (*ct*, 20.0), "ocellar-bristles" lacking (*ob*, 32.—), "vermilion" eye-color (*v*, 33.0), "tiny-bristles" (*tb*, 36.0), "miniature" wing-size (*m*, 36.1), "sable" body-color (*s*, 43.0), "garnet" eye-color (*g*, 44.4), "forked" bristles (*f*, 56.5). The tentative symbol for "vermilion-deficiency" is – (33. ±), for "vermilion-sable duplication" *V-S* (0.0), and for "sable-duplication" **S** (0.0). The symbol + is read "wild-type." Sable-duplication is equivalent to a wild-type allelomorph of sable situated at the extreme left end of the X; males of the constitution $\frac{\mathbf{S}}{s}$ are accordingly wild-type in appearance. Likewise *V-S* duplication "covers" the recessive genes vermilion and sable.

Origin and Lethal Action of Vermilion-Deficiency.

The recessive sex-linked mutation tiny-bristles had arisen in the early work on "sable-duplication" (unpublished data). It was not then convenient to give time to working out the locus of tiny-bristles

very accurately, so that a stock of tiny-bristles was carried along in the easiest way by breeding tiny-bristle males to their heterozygous sisters. Tiny-bristle females were found to be so infertile that they could not be depended upon. One of the parents of such a stock culture was a female carrying in one X chromosome tiny-bristles, garnet, and forked, and, in the other X, sable-duplication, sable, and garnet $\left(\frac{S\ \ s\ g}{tb\ \ g\ \ f}\right)$. The other parent was a male carrying sable-

TABLE I.

Offspring of Garnet Female, Constitution $\left(\frac{S\ \ s\ g}{tb\ \ g\ \ f}\right)$, *and Garnet Forked Male, Constitution* $(\underline{S\ \ g\ \ f})$.

Feb. 14, 1916.

Culture No.	Females.		Males.						
	g	*gf*	*g*	*tb gf*	*sg*	*tb sg*	*gf*	*tb g*	*sgf*
3,302	20	14	7	10	2	1	5	1	1

TABLE II.

Offspring of Garnet Female, Constitution $\left(\frac{S\ -\ g\ \ f}{S\ \ sg}\right)$, *and of Tiny-Bristle Garnet Forked Male* $(\underline{S\ \ tb\ g\ \ f})$.

Mar. 9, 1916.

Culture No.	Females.		Males.	
	g	*gf*	*g*	*gf*
3,680	51	57	44	11

duplication, garnet, and forked $(\underline{S\ \ g\ \ f})$. The offspring of this pair were of the expected kinds and frequencies (Table I). No lethal was present.

For the next generation a garnet female was crossed to a tiny-bristle garnet forked brother. The offspring showed that this male had received sable-duplication from the mother as the result of crossing over, and was of the constitution $\underline{S\ \ tb\ g\ \ f}$. The mother was expected to carry sable-duplication, sable, and garnet in one X and

sable-duplication, garnet, and forked in the other X $\left(\frac{\mathbf{S}\ \ s\ g}{\mathbf{S}\ \ g\ f}\right)$.

The progeny (Table II) agreed with the expectation except that in addition to these genes of the mother a new lethal had appeared which killed off half the males. The lethal gene had appeared by mutation in the $\underline{\mathbf{S}\ g\ f}$ X derived from the father, and its locus was 20 or more units from forked on the basis of the eleven crossovers in a total of 55 sons. This distance is great enough so that it was thought probable that the locus of the lethal was to the left of forked rather than further toward the distal end of the chromosome. No great importance was attached to this occurrence of a lethal, since they occur frequently; no less than five were detected in the early work on sable-duplication.

In the next generation a start was made to determine the locus of tiny-bristles, the slight information already in hand having indicated a position some 6 or 8 units to the left of sable; that is, in the neighborhood of miniature. It was thought more advisable to use vermilion before miniature, since there was some chance that the small size of the wings of the tiny-bristle mutation would interfere with the classification of the miniature wing character. Accordingly, some of the garnet daughters of Culture 3,680 were outcrossed to vermilion forked males. Eight of these daughters gave progeny in accordance with the constitution $\left(\frac{\mathbf{S}\ \ tb\ \ g\ \ f}{\mathbf{S}\ \ \ \ s\ g}\right)$ (Table III) which furnished data with respect to the amount of crossing over between tiny-bristle and forked.

However, two daughters (Table IV) gave results different in two respects: the lethal found present in the parental culture was here evidenced in the fact that there were only half as many males as females (86♂ : 181 ♀), and in the fact that the locus of this lethal corresponded to the position calculated for the lethal present in the previous generation, since its location was 19 units to the left of forked and about 2 units to the left of tiny-bristles. The mothers of the two cultures had come from eggs representing normal crossing over between the lethal and sable in Female 3,680. All the above results are normal; however, a totally unexpected feature appeared in the

fact that *half the females were vermilion although there had been no vermilion in the maternal ancestry.* The amount of crossing over between this vermilion and forked (19.9 per cent) was slightly less than the amount expected from ordinary vermilion and forked. But there was no crossing over observed between the vermilion and the lethal; that is, there were no vermilion sons. This set of facts immediately suggested that here was another case of "deficiency"[1]—

TABLE III.

Offspring of Eight Females, Constitution $\left(\frac{S \quad tb \quad g \quad f}{S \quad\quad s \; g}\right)$ *from Culture 3,680, and Vermilion Forked Males.*

Mar. 26, 1916.

	Females.	Males.				
		tb g f	*s*	*tb g*	*g f*	*v f*
Total.....	1,021	192	380	75	76	1 (XO)

TABLE IV.

Offspring of Two Garnet Daughters, Constitution $\left(\frac{S \; - \quad\quad s \; g}{S \quad tb \quad\quad g \quad f}\right)$ *from Culture 3,680, and Vermilion Forked Males.*

Mar. 26, 1916.

Culture No.	Females.				Males.		
	v l	*f*	*v f f*	+	*tb g f*	*g*	*tb g*
3,987	39	36	7	14	44	1	10
3,989	34	36	7	8	26	1	4
Total.....	73	72	14	22	70	2	14

that the process which gave rise to the lethal was not simple mutation but a process characterized by the "*inactivation*" *or loss of the genes of a whole section of the X chromosome.* The garnet forked father of Culture 3,302, according to this view, had produced a sperm whose X was "deficient" for the vermilion locus and for a section of chromosome long enough so that one or more genes necessary for the life of the male individual were rendered inoperative. The deficiency of

these vital genes had given rise to the lethal effect, and the deficiency for the vermilion locus rendered a female carrying one such chromosome virtually haploid for vermilion. A female, one of whose X chromosomes carries vermilion-deficiency and the other the recessive vermilion gene, shows the vermilion character as does a male carrying the vermilion gene in its single X chromosome. From the facts in certain other cases of deficiency, especially Notch 8 (N 8),[2] it is probable that the condition is not as simple as just represented, but that the vermilion-deficiency has some positive action, in some ways comparable to, but more extreme than, the action of the vermilion gene.

Linkage Tests of the Extent of the Deficient Region and the Disturbance in Crossing Over in Adjacent Regions.

The work with the first deficiency[1] had shown that there was probably no crossing over within the deficient region, and that the chromosome map was shortened by an amount equal to the length of the deficient region. In certain other cases of deficiency, notably dachs-deficiency,[3] it has been found that besides this shortening equivalent to the length of the deficient region there is extensive alteration in the amounts of crossing over in neighboring regions of the chromosome. This disturbance seems to take the form of a marked decrease in the amount of crossing over in the immediate vicinity of the deficiency with perhaps slight increases in more remote regions. Such an effect would follow from disturbed synaptic relations and would seem to argue that deficiency involves a real contraction of the deficient chromosome, with resultant "puckers" at synapsis, rather than merely an inactivation.

The first step taken was to test whether there was marked reduction of crossing over in the region to the left of the supposed deficiency. The vermilion forked and lethal forked crossover values had already shown that there was probably not much disturbance in the long region to the right of vermilion. The recessive mutation facet whose locus is at about 2.6 was used to control the left end.

[2] Mohr, O. L., *Genetics*, 1919, iv, in press.

[3] Bridges, C. B., and Morgan, T. H., *Carnegie Institution of Washington Publication, 278*, 1919, pt. ii.

Three of the vermilion daughters of Culture 3,987 were outcrossed to facet vermilion forked males (Table V).

The males of Table V showed that there was probably a decrease of crossing over immediately to the right of the deficient region, since the vermilion-deficiency sable crossover value was 7.4, instead of 10.0, which is the normal vermilion-sable value.

TABLE V.

Offspring of Vermilion Females, Constitution $\left(\frac{S - sg}{v \quad f}\right)$, *Outcrossed to Facet Vermilion Forked Males.*

Apr. 16, 1916.

Culture No.	Females.	Males.				
		vf	*vsg*	*vg*	*v*	*vsgf*
4,236	257	98	11	—	20	—
4,237	222	76	9	2	11	—
4,258	229	81	3	5	8	1
Total.....	708	255	23	7	39	1

TABLE VI.

Offspring of Vermilion Females, Constitution $\left(\frac{fa \quad v \quad f}{- \quad sg}\right)$ *from Table V, Outcrossed to Cut Vermilion Forked Males.*

May 8, 1916.

Culture No.	Females.	Males.					
		favf	*vf*	*favsg*	*fav*	*vsg*	*v*
4,514	43	8	2	1	2	—	—
4,518	78	22	7	—	2	—	—
4,519	61	17	4	—	3	1	3
Total.....	182	47	13	1	7	1	3

Over a dozen vermilion-deficient females whose father was facet vermilion forked were outcrossed to cut vermilion forked males, though only three of these cultures produced offspring (Table VI). The resulting data were rather insufficient, but indicated that there was probably somewhat less than the normal amount of crossing over

in the facet to deficiency interval. There was again considerable reduction in the crossing over between the deficiency and sable which was here only 2.8 instead of 10.0 per cent.

The cut vermilion forked male had been used in the mating just described in order that in the next generation a closer analysis of the crossover reduction immediately to the left of the deficiency might be made.

TABLE VII.

Offspring of Vermilion Females, Constitution $\left(\frac{ct\ \ v\ \ \ f}{-\ \ sg}\right)$.

June 28, 1916.

Culture No.	Females.	Males.						
		ct v f	*v f*	*ct v sg*	*ct v g*	*ct v*	*v*	*ct v sg f*
4,676	89	36	6	2	—	4	—	—
4,583	81	13	2	—	1	1	—	—
4,684	117	24	4	1	1	3	—	—
4,686	41	14	—	1	—	3	—	1
4,687	22	6	2	2	—	—	—	—
4,703	53	12	2	—	—	6	—	—
4,704	44	14	2	3	—	2	—	—
5,105	67	31	4	5	—	6	—	—
5,164	44	9	—	2	2	2	—	—
5,166	49	23	3	1	—	3	—	—
6,783	88	22	6	2	—	4	—	1
6,784	55	18	2	1	—	3	—	—
6,902	83	27	1	1	4	3	—	—
6,903	165	61	14	5	1	14	—	—
6,727	108	33	6	8	—	6	1	—
6,945	96	34	5	2	1	3	1	—
Total.....	1,202	377	59	36	10	63	2	2

Considerable data have been collected showing the amount of crossing over in females carrying cut vermilion and forked in one X chromosome and deficiency sable garnet in the other (Table VII).

The most significant crossover values calculable from the results of Table VII are cut deficiency which is 11.1 instead of 13.0—a decrease of 1.9 units, and deficiency sable which is 6.9 instead of 10.0—a decrease of 3.1 units.

In Table VIII is given a summary of the more significant crossover values as determined from all the experiments involving deficiency, with normal values for comparison.

From these values it is apparent that there is a reduction in crossing over between cut and vermilion-deficiency of about 2 units, while there is a similar reduction between deficiency and sable of about 3 units, or a total shortening of the chromosome map of about 5 units. It is known that not all the reduction to the right of deficiency is due to the length of the deficient region, since crossing over occurred between deficiency and tiny-bristles. Tiny-bristles and vermilion are normally 3.0 units apart, while there were two crossovers in a

TABLE VIII.

Comparison of Normal Crossover Values With Those Obtaining for Females Heterozygous for Vermilion-Deficiency.

Loci.	Deficiency.	Normal.	Change.
ct —	11.1	13.0	−1.9
— *tb*	2.3	3.0	−0.7
— *s*	6.8	10.0	−3.2
— *f*	20.1	22.9	−2.8
s g	1.8	1.4	+0.4
g f	12.4	12.1	+0.3

total of 86 males, which showed that about 2.3 per cent of crossing over occurs between deficiency and tiny-bristles. This evidence does not allow us to determine the actual length of the deficient region nor what proportion of the deficiency is to the left of the vermilion locus and how much is to the right. The garnet forked value is normal or perhaps slightly high.

The normal values used for comparison in Table VIII are the mean values derived from several experiments[4] and are not, as would have been more desirable, from sister cultures. While the two sets of values are therefore not strictly comparable, the differences observed were so constant in direction that there can be little doubt of the reality of the changes.

[4] Morgan, T. H., and Bridges, C. B., *Carnegie Institution of Washington, Publication No. 237*, 1916; also unpublished data.

Relation between Vermilion-Deficiency and Vermilion-Duplication.

That the deficient region actually does extend to the left of the vermilion locus was indicated by use of vermilion-duplication. The stock of vermilion-duplication consists of flies homozygous for the vermilion gene and at the same time homozygous for the not-vermilion gene, since these flies carry at the left end (point of spindle-fiber attachment?) a transposed section of chromosome including the loci for vermilion and sable. These flies are not vermilion in appearance since the two wild-type allelomorphs dominate over the two recessive vermilion genes. But if such females are crossed to vermilion males all the daughters are vermilion and all the sons are wild-type—simulating the Abraxas type of "criss-cross" inheritance, or dominance of vermilion. These daughters are vermilion because two recessive vermilion genes are present and dominate over the single wild-type allelomorphs of the F_1 female. When vermilion females carrying vermilion in one X and deficiency in the other were crossed to males of the duplication stock, all the daughters were vermilion. In its effects upon the dominance of vermilion, vermilion-deficiency may be substituted for a vermilion gene without distinguishable difference. This fact also indicates a positive action of vermilion-deficiency. Had the deficiency been neutral in effect, then the flies carrying vermilion, not-vermilion, and vermilion-deficiency should have been wild-type like the normal diploid heterozygote.

The F_1 females were crossed to males from the vermilion-duplication stock (Table IX). The most significant point observed in F_2 was that the lethal effect of vermilion-deficiency was not annulled by the presence of the duplication. The probable explanation of this is that the two regions do not coincide; *i.e.*, although the duplicating fragment includes the loci from vermilion to the right as far as sable, nevertheless it does not extend so far to the left of vermilion as the deficiency does. This excess of the deficient region to the left of vermilion would have to be enough to include one or more loci vital to the animal.

It should be noticed that there was 35.5 per cent of crossing over between zero and vermilion-deficiency, which is about 3.5 units more than the average normal valuc.

TABLE IX.

Offspring of Vermilion Females, Constitution $\left(\frac{V-S\ v}{-\ sg}\right)$, *and Wild-Type Males, Constitution* $(\frac{V-S\quad v}{\quad\quad\quad})$.

Apr. 23, 1917.

Culture No.	Females.		Males.				
	+	v	+	v	g	v sg	v g
7,055	79	86	37	20	4	1	1
7,065	76	75	32	26	5	—	—
7,076	19	47	13	8	3	—	—
7,080	31	34	18	8	3	—	—
7,081	59	53	34	21	7	—	—
Total.....	264	295	134	83	22	1	1

Haploid and Cytological Tests of the Extent of the Deficient Region.

Another method of getting light on the extent of the deficient region is by testing whether the deficiency works for other mutations in the same manner as with vermilion. Tiny-bristles and miniature were known by the linkage tests to be outside the region so that the failure of both to give pseudodominance was not unexpected. The only other mutant whose locus was close enough to be worth while testing was "ocellar-bristles" whose locus is to the left of vermilion by one or more units—not accurately located because of the poorness of the character. This mutant also failed to give pseudodominance so that its locus lies outside the deficient region.

Considerable cytological work was undertaken, but none of the slides were good enough technically to be able to demonstrate the deficiency cytologically.

Dominant Action of Deficiencies.

The stock of vermilion-deficiency was unusually difficult to maintain, being on the verge of extinction several times before its final loss. The same fact was observed with respect to the orginal forked-bar-deficiency and all the notch stocks, which have been lost (ex-

cept Notch 8) after a longer or shorter period of struggling existence. This fact also would indicate a positive action on the part of deficiency such that the heterozygous females are of lower viability, fertility, and productivity, even when, as in the cases of forked- and of vermilion-deficiencies, no somatic character is observable.

THE NATURE OF THE LATENT PERIOD IN THE PHOTIC RESPONSE OF MYA ARENARIA.

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I.

The response of *Mya* to light involves the existence of a latent period. The stimulus consists of the exposure of the animal to the influence of light for a short interval. Following the exposure period, *Mya* may remain in the dark for a length of time which is usually greater than the exposure. At the end of this latent period it retracts its double siphon in response to the stimulus. The demonstration of the latent period may be easily accomplished by the use of a photographic shutter. By this means the animal is exposed for very short intervals. At intensities above 3,000 meter candles, an exposure of 0.01 second is sufficient to produce a response after a latent period of about 1.3 seconds.

The sensitization process occurring during the exposure period has already been shown to depend on a reversible photochemical reaction (Hecht, 1919, *a*). This consists of the decomposition of a photosensitive substance into its two precursors. The dynamics of the decomposition and regeneration of the sensitive material follows closely the peculiarities of the sensory behavior of the animal.

The significance of the latent period, however, is still unknown. Of course it must include the time for the transmission of impulses, and for the latent period of the contraction of the muscles used in the siphon response. These are, however, notoriously short intervals of time, and may be almost ignored in the face of a latent period which lasts at least 1.3 seconds, and may be as long as 3 or 4 seconds under certain conditions. In order, therefore, that the photosensory process in *Mya* be understood in its details, it is necessary to investigate the probable nature of the latent period by a consideration of some of its properties.

II.

During the exposure of *Mya* to light, a photosensitive material is decomposed into its two precursors. If these precursor substances are in any way concerned with the nature of the latent period, there should exist some relation between the quantities in which they are produced and the duration of the latent period. Experiments were therefore performed to determine the influence of the exposure period on the length of the latent period.

The reaction time of *Mya* under given conditions is quite a constant quantity, and is well over 1 second in length. Any significant changes in its duration can, therefore, be measured by means of an ordinary stop-watch. On the other hand, the exposure periods necessary to produce a response are very small, and must be measured in a much finer manner. This was done by means of a camera shutter loaned to me by Dr. A. C. Redfield, to whom I am greatly indebted.

Seven exposures were selected for use. They were calibrated by photographing a small white spot moving in a circle at a definitely known rate in front of a dead black surface. The photographs were made at noon of a brilliantly sunlit day, using the largest stop of a lens about 4 cm. in diameter. Therefore the angle subtended by the streak image on the photographic plate may be considered an accurate measure of the duration of the exposure. The seven exposure periods were found to cover a range from 0.015 to 0.257 second; their values are given in Table I.

TABLE I.

Duration of Exposures Obtained from the Camera Shutter Employed in These Experiments.

Exposure.	Duration.
	sec.
1	0.015
2	0.078
3	0.133
4	0.176
5	0.206
6	0.233
7	0.257

For the experiments, animals were used which had been in the dark for some time,—more than long enough for them to have become thoroughly dark-adapted (Hecht, 1919, *a*). Observations were made by the dim light of a ruby lamp to which the animals are practically insensitive. The reaction time to lights of different intensities and for different exposure periods was determined for twenty-two individuals.

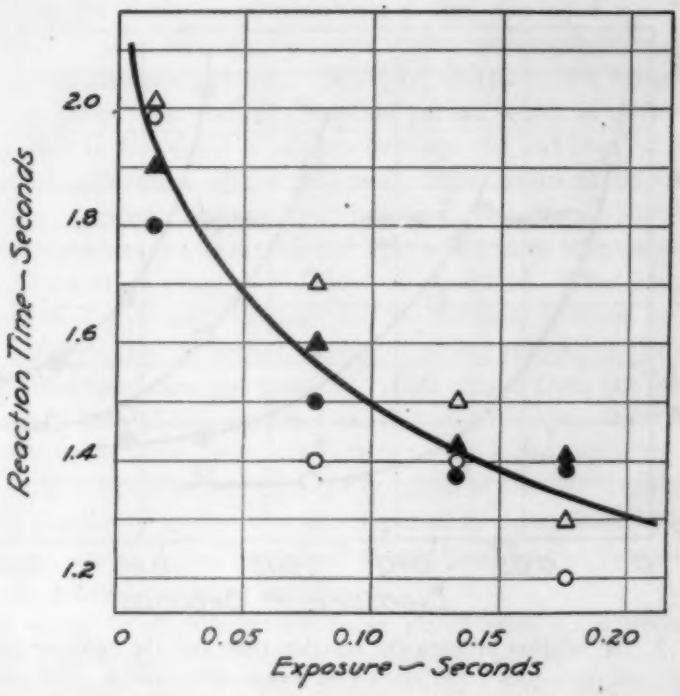

FIG. 1. Individual determinations of the reaction time of four animals for different exposure periods. The points read to the nearest tenth of a second. The curve joins the averages for the different exposures.

All the experiments agree in showing that for a given intensity the reaction time varies inversely with the exposure, for exposure periods shorter than the sensitization period. Exposures for intervals greater than the sensitization period make no change in the duration of the reaction time. The sensitization period may thus be defined

as the minimum exposure necessary to produce the minimum reaction time.

In Fig. 1 are given the detailed results of one experiment with four animals. Each point represents an individual determination of the reaction time at the exposure indicated. The solid line joins the averages of the four readings for each exposure period. The figure shows, that though the experimental error is large, the changes in the reaction time for different exposures are well beyond the varia-

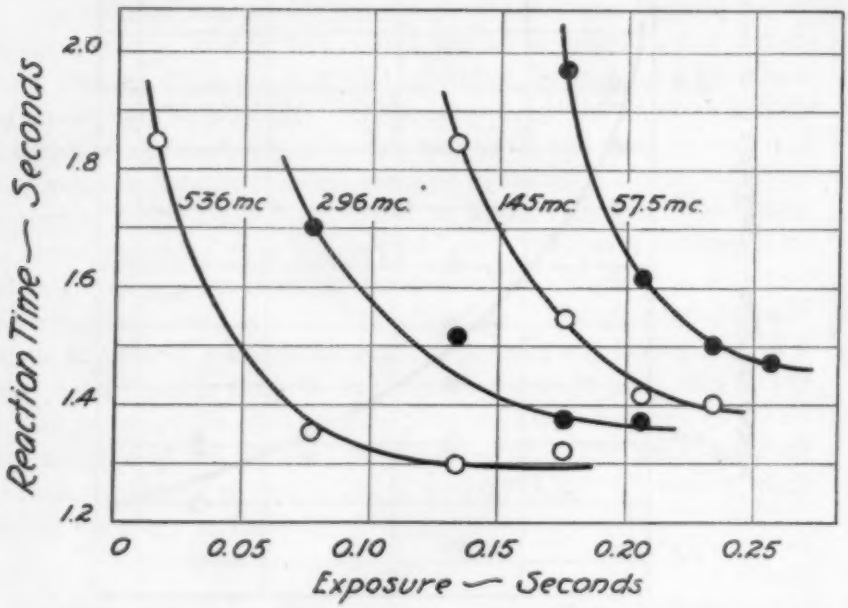

FIG. 2. The relation between the reaction time and the exposure period at four different intensities. The symbol *mc* means *meter candles*. The points are the averages of four determinations, one each on four animals.

tions due to the experimental error. Data for four other animals are recorded in the points of Fig. 2. Each point is the average of the reaction time of the four animals, one determination having been made on each animal for every exposure.

The trend of all the experiments is sufficiently clear from those presented in the two figures. They all show that the longer the exposure, the shorter is the reaction time. The difference between

the reaction time and the exposure period is the latent period. Therefore the latent period also varies inversely with the duration of the exposure. The suggestion that the latent period is in some way related to the formation of precursor materials is consequently justified.

III.

The latent period, being the interval during which *Mya* may remain in the dark, is certainly not a time during which "nothing happens." The fact that it varies under certain conditions is reason enough against such an idea. We may, therefore, rest assured that a process takes place during the latent period, which in some way is a vital link in the chain of events between the incidence of the light and the appearance of the response. There seem to be three possibilities for the nature of this process. The first is that the precursor substances formed by the light exert their stimulating action at a place other than where they are produced. The latent period would thus represent the time for the diffusion of these substances. A second possibility is that the latent period represents the duration of a subsidiary chemical reaction. This results from the interaction of one or both of the precursor substances with some other materials present in the sense organ. The time for the formation of the product of reaction would account for the duration of the latent period. The third possibility is that the latent period is determined by an independent chemical reaction which is catalyzed by the presence of the freshly formed precursor substances.

In order to decide among these possibilities it is necessary to inquire quantitatively into the relation between the exposure period and the latent period. Fig. 3 shows this for the experiment given in Fig. 1. Each point is the reciprocal of the average latent period plotted against the exposure time. That the points form a straight line is undoubted. Fig. 4 gives the reciprocals of the latent periods of the experiments presented in Fig. 2. In spite of the rather large observational error, it seems clear that the points may best be arranged in straight lines.

The exposure time measures the amount of photochemical activity. For these extremely short intervals, the amount of precursor ma-

terials formed by this photochemical activity may be considered directly proportional to the length of exposure. Since the reciprocal of the latent period represents the rate of the process which determines it, the data of Figs. 3 and 4 show that the velocity of the latent period process is a linear function of the concentration of freshly formed precursor materials.

This at once rules out the diffusion explanation. There is one case in which the rate of diffusion is a linear function of the con-

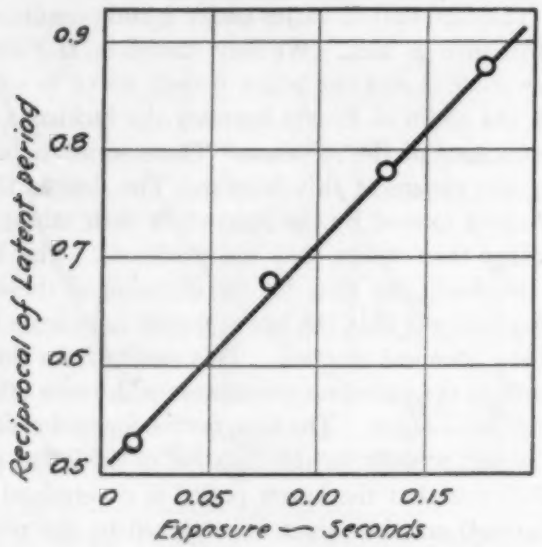

FIG. 3. The relation between the reciprocal of the latent period and the exposure period. The points are derived from the averages of the reaction time given in Fig. 1.

centration. This is when the concentration of diffused material is maintained at zero or nearly zero by continually removing the diffused substance, or by allowing the diffusion to proceed into a comparatively large volume. This is obviously not the situation here, because it is essential that the precursor substances accumulate in sufficient quantity to initiate a response. An additional objection to the diffusion idea is the temperature coefficient of the latent period. The latent period shows a value of $\mu = 19{,}680$ (Arrhenius,

1915, p. 49) which corresponds to a temperature coefficient of between 2.5 and 3.0 (Hecht, 1919, *b*). This temperature coefficient is much too high for a diffusion process, and corresponds well with those observed for ordinary chemical reactions.

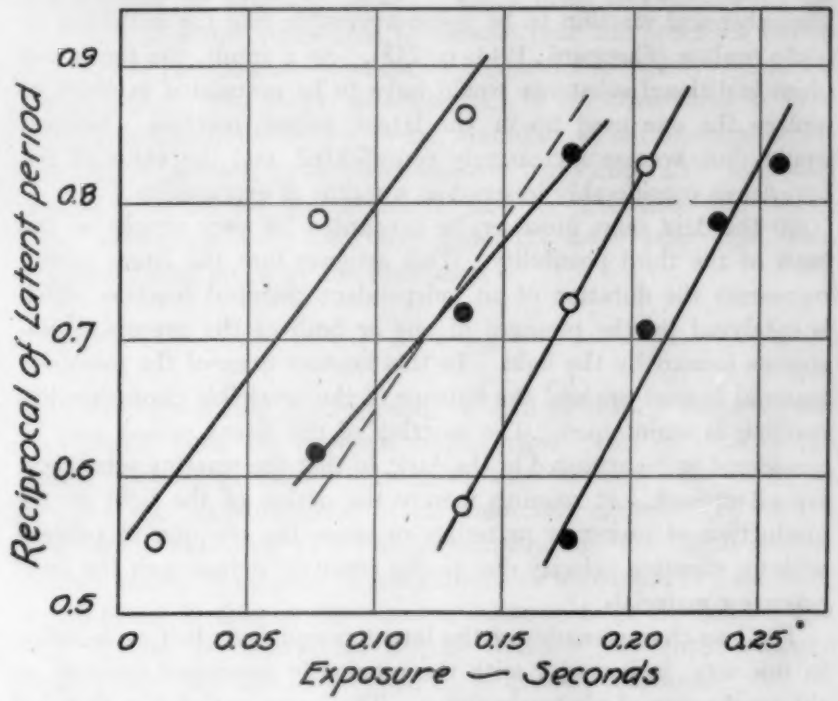

FIG. 4. The reciprocals of the latent period are derived from the data of Fig. 2. The interpretation is the same as that of Fig. 3. The broken line of the second series is drawn roughly parallel to the other three. It shows that the points of the second series may fit fairly well around the parallel line, thus indicating a fundamental similarity in the relationship at the different intensities. This rough parallelism is found in the data of the other animals of these experiments not recorded in the figures.

The data, at first sight, seem compatible with the idea of a subsidiary chemical reaction utilizing the precursor substances. This conception, however, involves an inconsistency, in that it requires the disappearance of the precursor materials during the latent period.

This means an upsetting of the balance in the reversible reaction system

$$S \rightleftharpoons P + A$$

previously established for the photic response of *Mya* (Hecht, 1919, *a*). Such a difficulty, however, might be surmounted by assuming the photochemical reaction to be pseudoreversible like the reduction of ferric oxalate (Sheppard, 1914, p. 213). As a result, the formation of an additional substance would have to be postulated in order to replace the one used up in the latent period reaction. Matters would thus become increasingly complicated, and the value of the hypothesis considerably lessened as a means of explanation.

All the data may, however, be accounted for very simply on the basis of the third possibility. This assumes that the latent period represents the duration of an independent chemical reaction which is catalyzed by the presence of one or both of the precursor substances formed by the light. In this manner none of the precursor material is used up, and the balance of the reversible photochemical reaction is maintained. The reaction of the latent period may be considered to be arranged in the dark, in that the reacting substances are all present. It requires merely the action of the light in the production of precursor materials to cause the reaction to proceed with an effective velocity due to the catalytic influence of the fresh precursor materials.

The two characteristics of the latent period find their explanation in this way, in harmony with the previously developed concepts of the mechanism of photosensitivity. The latent period is a chemical reaction; this agrees with the observed effect of the temperature (Hecht, 1919, *b*). The rate of the latent period process is a linear function of the amount of precursor substances formed during the exposure period. In explanation of this it will be remembered that it is a common characteristic of catalyzed reactions for the velocity of the reaction to be a linear function of the concentration of catalyst (Euler, 1912, p. 132).

IV.

· · In terms of the conception developed in the present paper, the latent period assumes a position of importance in the composition of the reaction time of *Mya* to light, coordinate with that of the sensitization or exposure period. There being no evidence to the contrary, it seems reasonable to assume that the processes underlying these two phases of the reaction time occur entirely within the sense organs. The events which happen in the sense organ of *Mya* when it is stimulated by light may, according to our findings, be expressed as follows. The photosensitive substance (S), originally formed from its two precursors (P and A,—Precursor and Accessory), is changed back into them under the influence of light, both reactions being given by the expression

$$S \underset{\text{"dark"}}{\overset{\text{light}}{\rightleftharpoons}} P + A.$$

This happens during the exposure to light or during the sensitization period when the exposure is prolonged. One or both of the freshly formed precursor substances then immediately serve to catalyze the transformation of an innocuous material (L) into a stimulating substance (T). This occurs during the latent period. When a sufficient amount of the stimulating substance (T) has been accumulated, it acts on the nervous connections to the sense organ and initiates the retraction of the siphon. The entire sensory process may therefore be summed up in the two reactions:

$$S \rightarrow P + A;\ L \,||\, P + A \,|| \rightarrow T$$

in which the symbol $||P + A||$ signifies catalysis by one or both of the precursor substances.

Two reactions related as are these correspond to a well known group of photochemical reactions classed by Weigert as "catalytic photochemical reactions in which the catalyst remains after the action of light." Among these are included so familiar a process as the exposure of a photographic plate and the development of the image under proper conditions (Weigert, 1911, p. 74).

The hypothesis of photoreception, here presented rather concretely, will undoubtedly have to be modified in one way or another

as the investigation of the photic sensitivity of *Mya* and other animals proceeds. The concreteness of the conception, however, should prove a useful tool in the acquisition of additional knowledge in this field.

SUMMARY.

The latent period in the response of *Mya* to illumination varies inversely as the duration of the exposure to which it is subjected. The reciprocal of the latent period, measuring the velocity of the process which underlies it, is a linear function of the exposure period. Since the duration of the exposure represents the amount of photochemical activity, it is concluded that the substances formed at that time act to catalyze a chemical reaction which determines the duration of the latent period.

This explanation is in accord with the previous work on the photochemical reaction and with the effect of temperature on the latent period. As a result of the combined investigations there is presented a concrete hypothesis for the mechanism of photic reception in *Mya*.

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THE EFFECT OF TEMPERATURE ON THE LATENT PERIOD IN THE PHOTIC RESPONSE OF MYA ARENARIA.

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I.

The purpose of this paper is to present an analysis of the relation between the temperature and the duration of a biological process. The numerous investigations which have already been published (Kanitz, 1915) have demonstrated that biological activities exhibit variations in rate at different temperatures. Usually the speed of the process is an exponential function of the temperature. Occasionally the relation between the two is linear. The latter phase has been particularly emphasized by Krogh (1914) and his colleagues.

However, no matter what the relation between the temperature and the rate may be, it fails to hold at higher temperatures. The rate of increase of the activity falls off decidedly. The velocity of the process soon reaches a maximum at a critical temperature. Above this so called optimum the activity declines or ceases altogether.

There has been much discussion as to the significance of these variations (Kanitz, 1915), and of the magnitudes of Q_{10}, the temperature coefficient for 10°C. On the one hand, the value of the temperature coefficient, as usually found between 2 and 3, has been made to signify that the fundamental process underlying the activity is a chemical reaction. This is because of the well known van't Hoff rule for the relation between the temperature and the velocity of ordinary chemical reactions. On the other hand, much effort has been spent to show that biological processes do not possess a constant temperature coefficient even for temperatures below the optimum (Krogh, 1914), and that consequently they do not obey the law

of van't Hoff. The deviations above the optimum temperature are generally dismissed by attributing them to the destructive effect of heat on protoplasm.

As has been pointed out (Snyder, 1911), the lack of constancy of the temperature coefficient is no bar to a process being chemical in nature. This is simply because purely chemical reactions also fail to show a constant temperature coefficient. The emphasis is to be placed on the order of magnitude of the coefficient and not on its invariability.

The real difficulty, however, is that biological phenomena do not represent single chemical reactions. In order, therefore, that the variations shown by biological activities be properly understood, it is necessary to know the nature and number of the chemical reactions concerned, and also their interrelations. Osterhout (1917) has indeed emphasized this by assuming a wholly imaginary biological activity composed of two consecutive chemical reactions, and synthesizing the effect of temperature on their combined activity. An analysis of data *actually secured* in the study of a vital process has apparently never been made in any but the most approximate manner. This is especially true of the variations exhibited by vital processes at higher temperatures.

Aside from the work of Blackman (1905), so vigorously criticized by Kanitz (1915, p. 22), the one significant contribution in this direction has been made by Pütter (1914). In the effect of temperature on the oxygen consumption of the leech, Pütter attributes the usual variations at higher temperatures to an increasing effect of an interfering process. For the estimation of this second factor, Pütter compares his actual results with those calculated on the basis of a constant temperature coefficient. The difference between the two is then shown to possess an approximately constant temperature coefficient of its own.

Pütter's idea, though correct in general conception, does not yield a quantitatively correct analysis. One reason for this is, as he realizes himself, that his data are not comparable at different temperatures; he measures the amount of oxygen consumed in a given time instead of the time for the consumption of a given amount of oxygen. In addition, we are wholly ignorant of the chemical and physical reactions involved in the process. Without such knowl-

edge it is impossible to apply the mathematical reasoning which is a prime essential for the proper comprehension of the data. The general idea, however, is a decidedly fruitful one; and, though arrived at independently, the analysis to be given in the present paper is really a quantitative application of just such a conception as developed by Pütter. The same idea has already shown its possibilities in the investigations of Tammann (1895) and of Duclaux (1899) on the effect of temperature on the activity of enzymes.

The case to be considered is the photic sensitivity of the mollusk *Mya arenaria*. Previous work (Hecht, 1919, *a*, *b*) has enabled us to propose an hypothesis to account for the photic behavior of this animal. The essential value of this hypothesis is its assumption of definite chemical reactions, the dynamics of which are known, and the interrelations of which are given. The effect of temperature on the photic behavior of *Mya* should therefore permit of a quantitative analysis in terms of the suggested hypothesis. The success of this analysis will then reciprocally furnish justification for the acceptance of the hypothesis.

II.

Mya responds to illumination by a rapid retraction of its siphons. Its reaction time is composed of two parts. The first is a sensitization period. This is the smallest interval of time during which the animal must remain exposed to the light in order to respond at the end of the usual reaction time. The second is a latent period during which *Mya* may remain in the dark. At the end of this period, the organism retracts its siphons exactly as if it had been exposed for the entire reaction time.

The sensitization period represents the duration of a photochemical reaction. This has been shown (Hecht, 1919, *a*) to consist of the decomposition of a photosensitive substance (S) into its two precursors (P and A), according to the reversible system

$$S \underset{\text{"dark"}}{\overset{\text{light}}{\rightleftharpoons}} P + A. \quad (1)$$

The duration of the latent period is determined by the velocity of an independent chemical reaction

$$L \rightarrow T \quad (2)$$

in which an innocuous substance (L) is changed into the stimulating substance (T). The activity of this reaction is catalyzed by the presence of one or both of the precursor materials (P and A) freshly formed during the sensitization period (Hecht, 1919, *b*). A slight fraction of the latent period represents the time for the conduction of impulses. This process, however, is very rapid, and may be entirely discounted in the comparatively large magnitudes of time concerned in the latent period.

The effect of temperature changes on the reaction time of *Mya* can therefore be considered as causing changes in the velocity of a photochemical reaction (1) and of an ordinary chemical reaction (2). It is well known that photochemical reactions suffer but slight changes with an alteration in the temperature. In fact, the sensitization process in *Mya* representing this photochemical reaction varies but slightly with the temperature. Data to be presented elsewhere give its temperature coefficient for 10°C. to be about 1.3 or less. The latent period, however, representing an ordinary chemical reaction varies decidedly with the temperature. This may be demonstrated by determining the latent period at different temperatures as a result of a constant exposure period. Fig. 1 gives the data of one such experiment in which the exposure was for 0.078 second to an intensity of 400 meter candles. The latent period is the difference between the total reaction time and the exposure period of 0.078 second. It is therefore clear that the bulk of the variation in the reaction time resulting from a change in temperature is due to the alteration in the latent period. Consequently, by subtracting the sensitization period from the reaction time determined as a whole, it is possible to measure the exact relation between the temperature and the duration of the latent period.

The experiments thus resolve themselves into measuring at different temperatures the reaction time of an animal to a single intensity of light. Preliminary experiments showed that about 40 meter candles is the right intensity to use. This gives a reaction

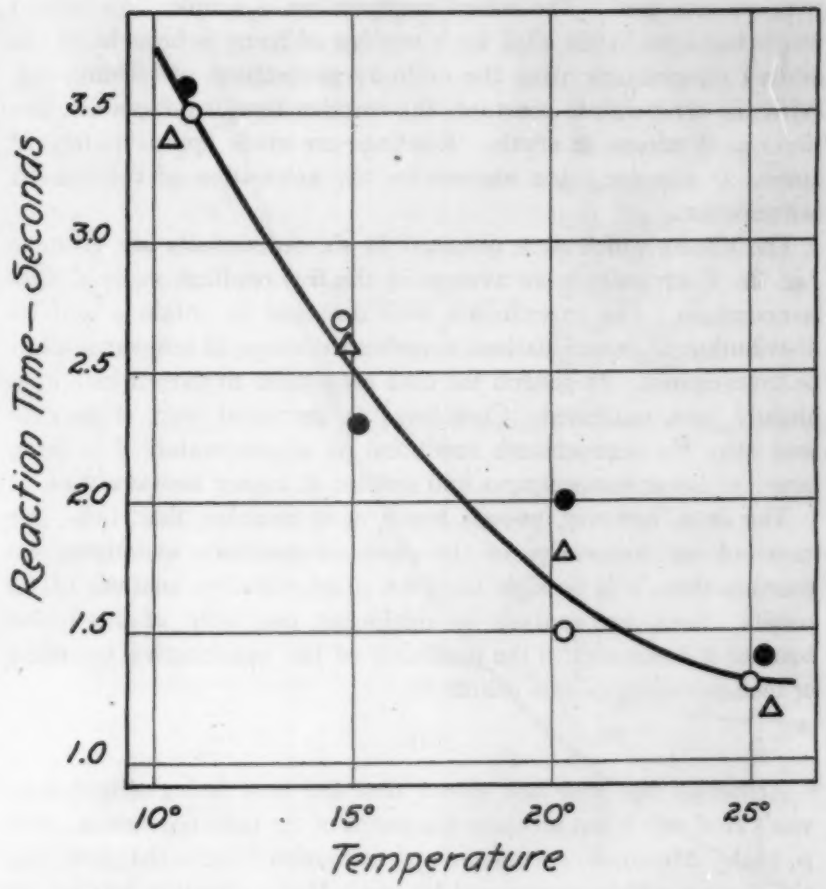

FIG. 1. Temperature and latent period. The animals were exposed to an intensity of 400 meter candles for 0.078 second by means of a camera shutter. The latent period is the time during which the animals were actually in the dark. Since the exposure is so very short, the reaction time as measured with a stop-watch may be considered entirely as the latent period. The points represent single, individual readings for the three animals of this experiment.

time sufficiently large to be measured comfortably. In addition, the sensitization period is quite small, averaging 0.21 second for the temperatures used. The actual manipulation is simple. An animal which has been in the dark for a number of hours is brought to the desired temperature using the ordinary precautions of stirring, etc. With the temperature constant, the reaction time is determined five times at 5 minute intervals. Readings are made approximately 5° apart, 20 minutes being allowed for the acquisition of the desired temperature.

The results which were obtained in six experiments are given in Fig. 2. Each point is an average of the five readings made at that temperature. The experiments were arranged to obtain a uniform distribution of determinations covering the range of temperatures to be investigated. In general the data are similar to those which have already been published. Considered in the usual way, it may be said that the temperature coefficient is approximately 2.5, being larger at lower temperatures and smaller at higher temperatures.

The data, however, possess much more meaning than this. Because of our knowledge of the chemical reactions underlying the reaction time, it is possible to make a quantitative analysis of the results. Such an analysis is significant not only of itself, but because it demonstrates the possibility of the quantitative treatment of biological data of this character.

III.

Arrhenius has long ago shown that the now biologically famous van't Hoff rule is not an exact statement of the facts (Arrhenius, 1912, p. 124). Moreover, even as an approximation it lacks the theoretical significance which is possessed by van't Hoff's equation relating the temperature and the equilibrium constant of a chemical reaction. Arrhenius has therefore derived, as a special case of the latter relation, an equation which relates the velocity constant of a chemical reaction with the temperature. If k' and k'' are the velocity constants at the absolute temperatures T' and T'', this equation of Arrhenius states that

$$\frac{k''}{k'} = e^{\frac{\mu}{R}\left(\frac{1}{T'} - \frac{1}{T''}\right)} \qquad (3)$$

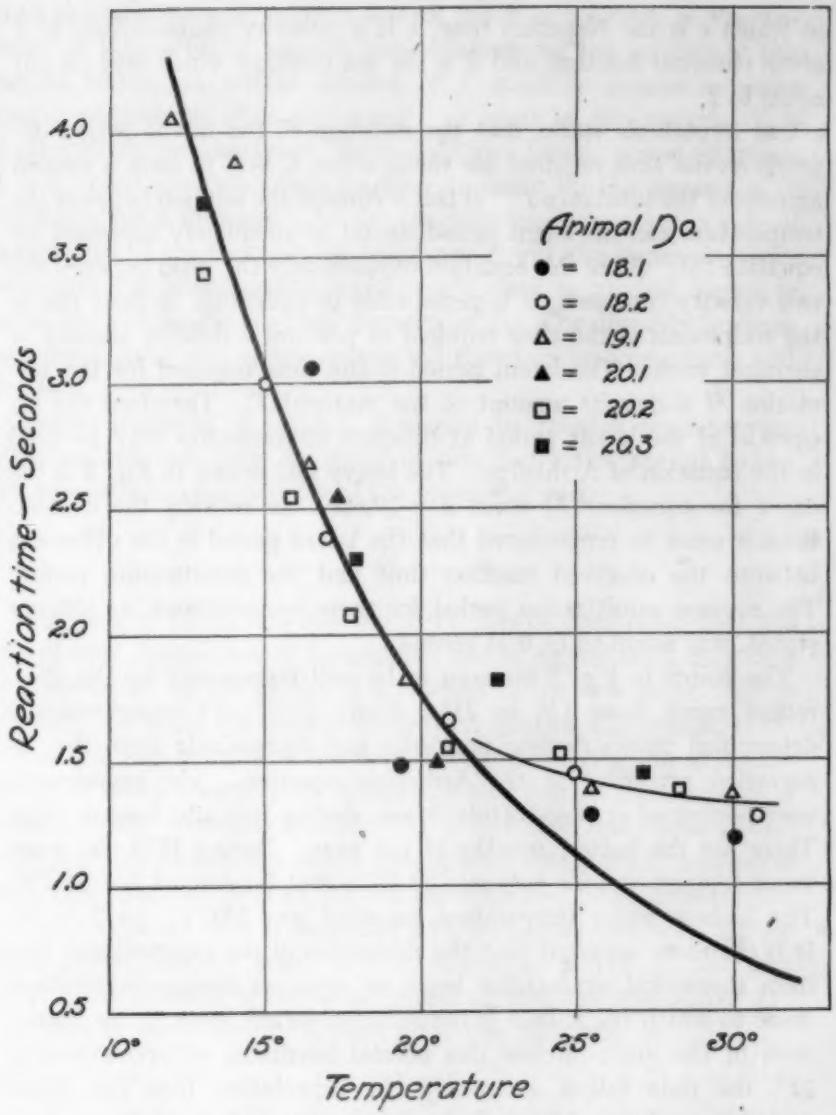

FIG. 2. Relation between temperature and reaction time. The reaction time minus 0.21 second gives the latent period. Each point is the average of five determinations on one animal. The heavy line is the theoretical expectation according to the Arrhenius formula when $\mu = 19{,}680$. The light line gives the general trend of the data above 21°.

in which e is the Naperian base, μ is a quantity characteristic of a given chemical reaction, and R is the gas constant which may be put equal to 2.

Our hypothesis states that the duration of the latent period depends on the time required for the reaction $L \rightarrow T$ to form a certain amount of the substance T. If this is correct, the relation between the temperature and the latent period should be adequately expressed by equation (3). Since this equation requires only the ratio between the two velocity constants, it is permissible to substitute in their places the reciprocals of the time required to perform a definite amount of chemical work. The latent period is the time required for the formation of a definite amount of the material T. Therefore the reciprocal of the latent period at different temperatures may be used in the equation of Arrhenius. The heavy line drawn in Fig. 2 is the curve for equation (3) when $\mu = 19{,}680$. In making the calculations it must be remembered that the latent period is the difference between the observed reaction time and the sensitization period. The average sensitization period for these temperatures, as already stated, was found to be 0.21 second.

The points in Fig. 2 are seen to be well represented by the theoretical curve from 13° to 21°. Above 21°C. the experimentally determined values deviate decidedly and increasingly from the expectation according to the Arrhenius equation. The experiments were performed at Woods Hole, Mass., during July and August, 1918. These are the hottest months of the year. During 1918 the mean water temperature for July was 20.2°C.; that for August was 21.7°C. The highest water temperature recorded was 23.0°C. for July 30. It is therefore apparent that the deviations of the experimental data from theoretical expectation begin to occur at temperatures above those to which the animal is normally subjected, even in the hottest days of the year. Below this normal maximum of approximately 21°, the data follow accurately the expectation that the latent period is conditioned by a single, simple chemical reaction.

The increasingly greater difference at higher temperatures between the calculated curve and the experimental data indicates clearly that a second factor has entered, the effect of which becomes more and more patent as the temperature increases. If we assume

that the substance T, formed during the latent period, is thermolabile, it is possible to account quantitatively for the activity of this second factor. A certain amount of T must be formed in order that a response of *Mya* may result. If some of this material T is destroyed at temperatures above 21°, the latent period reaction, $L \rightarrow T$, must proceed longer in order to make up the amount of T necessary for a response. As the temperature increases, the rate of destruction of the thermolabile substance T will also increase. Consequently the latent period reaction will have to proceed longer in order to make up the required quantity of T. The disparity between calculated curve and actual results will therefore become greater and greater.

If this reasoning is correct, it should be possible to determine quantitatively not only the effect of this destructive second factor, but the nature and dynamics of the process as well.

IV.

The work of Chick and Martin (1911) has demonstrated that the rate of heat coagulation of hemoglobin and of egg albumin may best be represented by the course of a reaction of the first order. More pertinent, perhaps, are the experiments of Madsen (Arrhenius, 1915) in which it was found that the spontaneous decomposition or inactivation of many thermolabile substances also follows the course of a monomolecular chemical reaction. It is therefore reasonable to assume that the destruction of our thermolabile substance T into some ineffective material N also follows the course of a monomolecular reaction. For the sake of simplicity let it be further assumed that the latent period reaction itself, $L \rightarrow T$, is also a reaction of the first order. The duration of the latent period at temperatures above 21° may thus be considered to depend upon two reactions,

$$L \rightarrow T, \; T \rightarrow N$$

forming a catenary system. From this reaction system a certain quantity of the substance T must accumulate in the sense organ before a response on the part of *Mya* can result.

The quantitative relations of these two reactions may be illustrated

graphically. In Fig. 3 are drawn the isotherms of the reaction $L \rightarrow T$ at the temperatures indicated. In the construction of these isotherms the quantity of L present at the beginning of the latent period is considered to be 1 gram-molecule, and the amount of T necessary to accumulate in the sense organ is put equal to 0.10 gram-molecule. These quantities are chosen arbitrarily for convenience. They may be put at any value without changing the analysis in the slightest degree. The essential point is merely that the amount at the beginning and the amount necessary for the response must be the same at all temperatures. Accepting these quantities and knowing

TABLE I.

Velocity Constants of the Latent Period Reaction, $L \rightarrow T$, at Different Temperatures. Values above 21° Calculated from the Arrhenius Equation when $\mu = 19{,}680$.

Temperature.	k_1
°C.	
13	0.0297
15	0.0376
17	0.0477
19	0.0605
21	0.0768
23	0.0948
25	0.117
27	0.144
29	0.178
30	0.215

the time interval during which the 0.10 mol of T is formed, it is a simple matter to calculate the velocity constants (k_1) of the latent period reaction from the equation

$$k_1 = \frac{2.303}{t} \log \frac{a}{a - x} \qquad (4)$$

which represents the course of a monomolecular reaction. In equation (4), t is equal to the latent period as given by the heavy line in Fig. 2; a is equal to 1 mol; x is 0.10 mol; and 2.303 converts Briggsian logarithms to the Naperian system. The velocity constants so obtained are given in Table I.

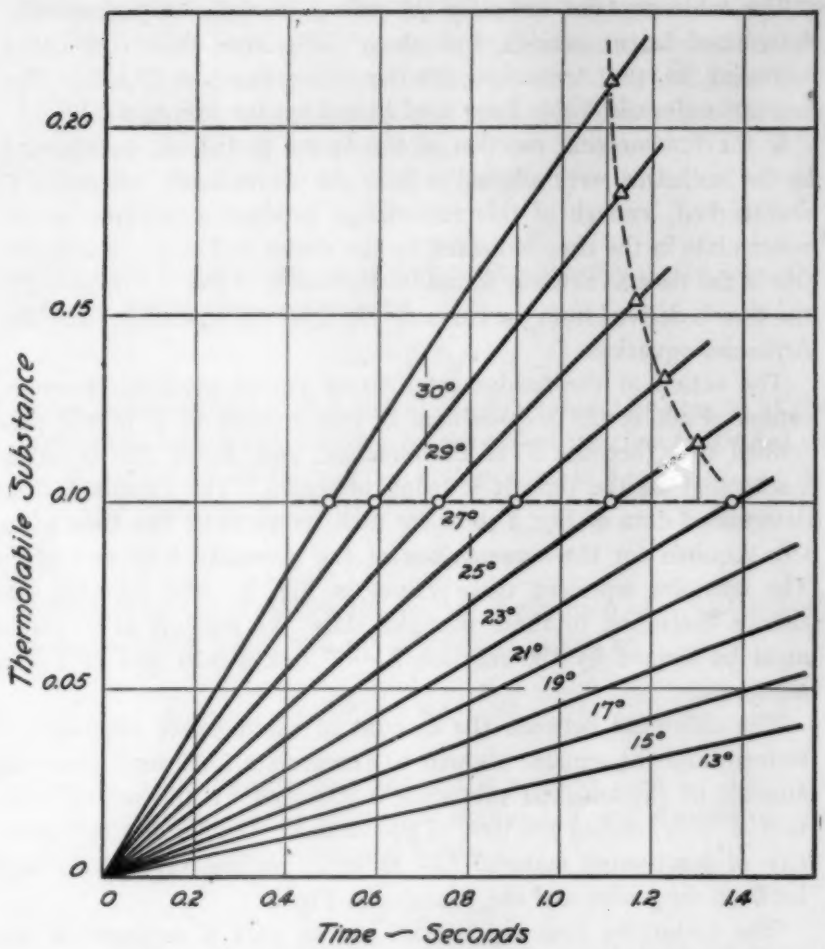

FIG. 3. Isotherms of the fundamental reaction, $L \rightarrow T$, of the latent period. Up to 21°, the reaction can accumulate without interference 0.10 mol of thermolabile substance in order to produce a response. Above 21°, the thermolabile substance is inactivated. The circles represent the time theoretically necessary to produce 0.10 mol *unhampered*. The triangles show the time actually required to produce enough thermolabile substance so that in spite of its inactivation there will still accumulate 0.10 mol of it in the sense organ. The vertical distance between the circles and the triangles gives the amount of thermolabile substance inactivated.

The table contains not only the values from the experimentally determined latent periods, but above 21°, also those calculated according to the Arrhenius equation (3) using $\mu = 19{,}680$. The constants given in Table I are used in making the curves of Fig. 3.

If the fundamental reaction of the latent period, as represented by the isotherms, were allowed to form the thermolabile substance T undisturbed, enough of this material to produce a response would accumulate in the time indicated by the circles in Fig. 3. Below 21° this is the time as actually found in the results of Fig. 2. Above 21° the time is derived from the curve of Fig. 2 by extrapolation from the Arrhenius equation.

The action of the fundamental latent period reaction, however, cannot result in the *accumulation* of this amount of T in the prescribed time, because T is thermolabile, and above 21° is being inactivated all the time it is being produced. The experimentally determined data of Fig. 2 show for each temperature the time actually required for the *accumulation* of the necessary 0.10 mol of T. The triangles represent these values in Fig. 3. The reaction isotherms extending to these triangles show the amount of T which must be formed by the reaction $L \rightarrow T$ before 0.10 mol of T can accumulate.

The difference between the amount of thermolabile substance T formed, and the amount allowed to accumulate (0.10 mol) gives the amount of thermolabile substance inactivated by the second reaction, $T \rightarrow N$, during the time of production. Graphically the quantity of inactivated material (N) is given by the vertical distance between the circles and the triangles in Fig. 3.

The quantities thus represented are in part a measure of the activity of the inactivating reaction $T \rightarrow N$. By no means, however, are they to be considered as the *direct* measure of this reaction, because the amount inactivated by the secondary reaction depends on one other condition besides its own speed. This other condition is the velocity of the fundamental reaction $L \rightarrow T$ which furnishes the pabulum for the secondary reaction. It is precisely this error of assuming the difference to be the *direct* measure of the inactivation factor, which vitiates the quantitative character of Pütter's (1914) explanation, as well as of the preliminary analysis given of the pres-

ent results (Hecht, 1919, *c*). In order to determine exactly the velocity of the inactivating reaction, it is necessary to utilize the dynamics of catenary reactions. This process does not lend itself readily to graphic representation, and must therefore be studied from certain mathematical considerations.

V.

We have to determine what happens in the case of the two consecutive monomolecular reactions

$$L \rightarrow T,\ T \rightarrow N.$$

It has been assumed that 1 mol of the substance L is present at the beginning of the latent period. At the end of the time t occupied by the latent period, let the reaction system contain x mols of L, y mols of T, and z mols of N. Therefore

$$x + y + z = 1. \quad (5)$$

According to the mass law the rate of diminution of L will depend on its concentration x, and will proceed according to

$$-\frac{dx}{dt} = k_1 x \quad (6)$$

where k_1 is the velocity constant of the reaction $L \rightarrow T$. The rate of the formation of the indifferent material N will depend on the concentration y of the substance T, and is

$$\frac{dz}{dt} = k_2 y \quad (7)$$

where k_2 denotes the velocity constant of the transformation of T to N. Therefore the rate at which T will *accumulate* in the sense organ will evidently be the difference between the rate of diminution of L and the rate of formation of N; in other words,

$$\frac{dy}{dt} = k_1 x - k_2 y. \quad (8)$$

The speed of the chemical system $L \rightarrow T \rightarrow N$ is fully determined by these three simultaneous differential equations. The conditions

which govern the behavior of the four variables in the equations are well known, and have been subjected to experimental verification (Mellor, 1916, p. 434). Without going into the details of the mathematics of the matter, it is sufficient to say that by combining equations (5), (6), (7), and (8), and integrating under proper conditions, an equation is deduced which gives the amount z of the substance N formed in a given time t in the terms of the two velocity constants k_1 and k_2. This equation is

$$z - 1 = \frac{k_2}{k_1 - k_2} \cdot \frac{1}{e^{k_1 t}} - \frac{k_1}{k_1 - k_2} \cdot \frac{1}{e^{k_2 t}} \qquad (9)$$

in which all the terms have their previous significance, and e is, as usual, the Naperian base.

TABLE II.

Velocity Constants of the Fundamental Reaction of the Latent Period, and of the Inactivation Reaction. Amount of Thermolabile Substance Inactivated above 21°, and the Time (t) during Which the Inactivation Actually Takes Place.

Temperature.	Latent period. (t)	k_1	k_2	z
°C.	*sec.*			*mol*
23	1.30	0.0948	0.239	0.0161
25	1.22	0.117	0.500	0.0332
27	1.16	0.144	0.826	0.0540
29	1.13	0.178	1.22	0.0824
30	1.11	0.215	1.63	0.1125

We already know the values of t, the actual latent period; these are given by the experimentally determined points of Fig. 2 and in part by the triangles in Fig. 3. The amount of N present in the system is the quantity z of thermolabile substance T which has been inactivated. This is also known from Fig. 3, and its value at different temperatures is given in Table II. Also, k_1 is known, its values having been given in Table I. The only unknown in equation (9), therefore, is k_2, the velocity constant of the inactivating reaction $T \rightarrow N$. Equation (9) may then be solved for k_2, which will give us precisely the information we desire: the values of this velocity con-

stant at the different temperatures. Computation of equation (9) using the various values of t, k_1, and z yields the quantities plotted in Fig. 4, and given in Table II.

We are now in possession of the means of estimating the relation between the temperature and the speed of the inactivating reaction $T \rightarrow N$. If our reasoning has been correct, these velocity constants should be related to one another according to the Arrhenius equation (3) previously given. Fig. 4 shows this to be true. The points are the logarithms of the velocity constants k_2, whereas the curve gives the theoretical expectation according to equation (3) solved for $\log k_2$ as follows:

$$\log k_2'' = \log k_2' + \left[\frac{1}{2.303} \cdot \frac{\mu}{R}\left(\frac{1}{T'} - \frac{1}{T''}\right)\right] \qquad (10)$$

In making the computations, T' is put at 296° (=23.0°C.) and k_2' is the value of k_2 at this temperature as given in Fig. 4. The factor $\frac{1}{2.303}$ converts natural into common logarithms. For drawing the curve in Fig. 4, μ is equal to 48,500. A much better agreement between observed points and the theoretical curve is hardly to be expected under the circumstances.

The fact that the hypothetical inactivation reaction, $T \rightarrow N$, shows a constant value of μ brings increased confidence in the reasons for its assumption. More convincing, however, is the order of magnitude of μ. In the table collected by Arrhenius (1915, p. 54) it is shown that ordinary chemical reactions such as saponifications and hydrolyses possess values of μ between 10,000 and 20,000. This agrees well with our findings of μ = 19,680 for the fundamental reaction of the latent period, $L \rightarrow T$. However, the chemical reactions involved in spontaneous destructions and in heat coagulations have values of μ which are rarely below 30,000, and are usually well above this figure. For the destruction of trypsin, μ = 62,000; for the heat coagulation of hemoglobin, μ = 60,100; for the inactivation of emulsion, μ = 45,000; etc. It is therefore highly significant that the reaction $T \rightarrow N$, postulated for the heat inactivation of the thermolabile substance T, shows a value of μ = 48,500, thoroughly in accord with those usually found for such processes.

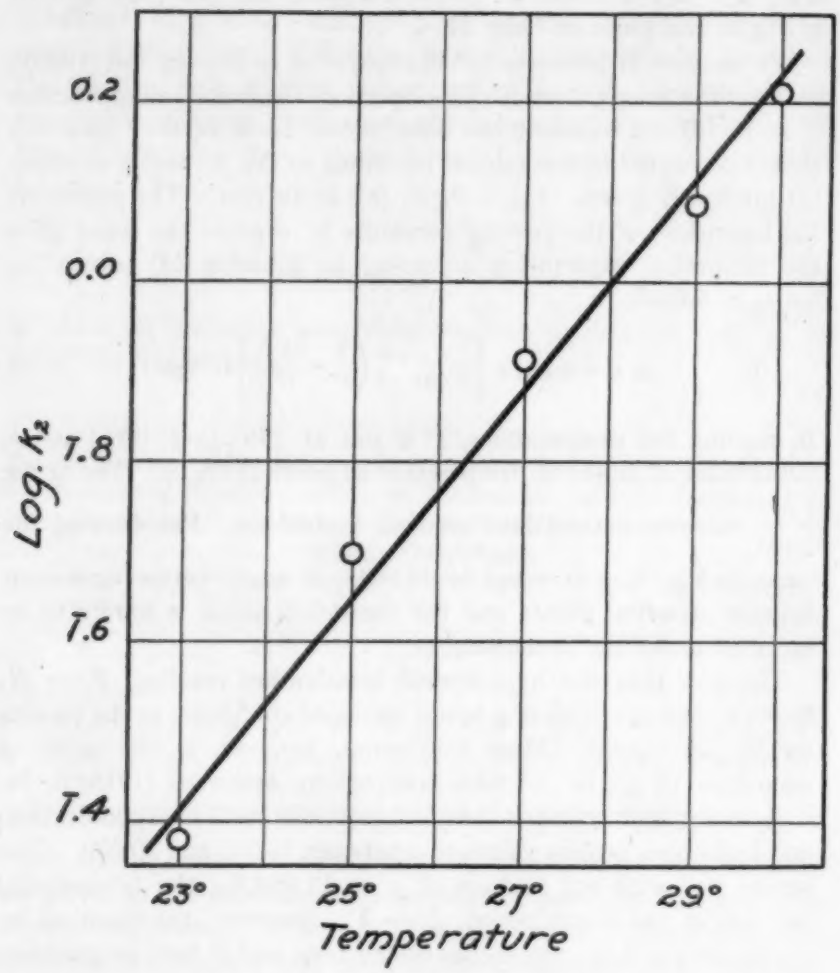

FIG. 4. Relation between the temperature and the velocity constants of the inactivation reaction $T \rightarrow N$. The points are those computed from the experimental data. The curve is the theoretical expectation from the Arrhenius equation when $\mu = 48{,}500$.

VI.

In considering the results of this analysis, it is necessary to reiterate a caution previously mentioned. This concerns the amounts of L and of T assumed in the quantitative treatment of the data. The assumption of 1 mol of L at the beginning of the latent period, and of 0.10 mol. of T at the end, in no way affects the analysis except in so far as it makes it possible to present the matter graphically. The actual quantities may be those represented, or, what is more probable, they may not. The results of the analysis, however, will be the same, as long as we accept the fundamental idea that a definite amount of the thermolabile substance T must accumulate in the sense organ in order to produce the inner stimulus for retraction of the siphons.

Considered in such general terms, the relation of the latent period to the temperature may be stated as follows. The duration of the latent period depends fundamentally on the accumulation of a certain amount of material as the result of a single, simple chemical reaction. The variations of this reaction with the temperature are adequately expressed by the Arrhenius equation (3) when $\mu = 19{,}680$. At temperatures above 21°C., however, this substance which accumulates in the sense organ is perceptibly inactivated by heat. The reaction expressing this inactivation is of the kind usually found for spontaneous destructions, and its relation to the temperature is also adequately expressed by the Arrhenius equation when $\mu = 48{,}500$.

We therefore have a destruction reaction, the velocity of which increases more than twice as rapidly with the temperature as does the fundamental reaction which produces the thermolabile substance. At temperatures slightly above 21°, a balance may be struck between destruction and production so that sufficient thermolabile substance will eventually accumulate to produce a response. Soon, however, the destruction reaction is more than fast enough to inactivate the thermolabile material as rapidly as it is formed. The result is that no thermolabile substance can accumulate in the sense organ. In *Mya* this happens at temperatures above 35°C., when no amount of exposure to light can result in a retraction of its siphons.

VII.

The analysis with which we have been occupied, though it accounts for the data in a simple way, is not the only one possible. Even in the terms of the hypothesis of photoreception which has been adopted for *Mya*, there is at least one other explanation, just as plausible but not as simple, which deserves to be mentioned. Without entering into details, I wish to present this alternative analysis in its barest outlines.

The reaction of the latent period, $L \rightarrow T$, is catalyzed by the precursor material freshly formed during the sensitization period. Organic catalysts are enzymes, and enzymes are notoriously thermolabile. Since the velocity of the latent period reaction is a linear function of the concentration of catalytic precursor (Hecht, 1919, *b*), the deviations of the latent period at higher temperatures may be considered as due to the destruction of some of this catalytic agent. The velocity of the latent period reaction becomes slower because of the decrease of catalyst. The time required to form a definite amount of T to produce a response is, therefore, lengthened beyond that required on the basis of the Arrhenius equation (3). Assuming that the destruction of the precursor catalyst follows the usual course of such spontaneous decompositions, it is possible to estimate quantitatively its velocity constant, and to express the entire process diagrammatically.

The actual mathematical considerations, however, are rather involved. The destruction of the precursor material must be estimated not only during the latent period after its production, but during the sensitization period while it is still being produced. The simpler analysis has, therefore, been given in detail because it fits the facts just as well. Certain deductions from the two explanations are, however, different for the two cases, and further experimentation will show which is more probably correct.

SUMMARY.

1. The effect of temperature on the reaction time of *Mya* to light is mainly confined to the latent period. The sensitization period, representing a photochemical process, is changed comparatively little.

2. The relation between the latent period and the temperature is adequately expressed by the Arrhenius equation, for temperatures below 21°C. Above this temperature, the latent period becomes increasingly longer than is required by the Arrhenius formula when $\mu = 19{,}680$.

3. These deviations, occurring above the highest environmental temperature of *Mya*, are explained on the assumption that the principal product formed during the latent period is inactivated by heat.

4. Calculation of the velocity of the hypothetical inactivation reaction at different temperatures shows that it also follows the Arrhenius rule when $\mu = 48{,}500$. This value of μ corresponds to those generally found for spontaneous inactivations and destructions.

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THE PHYSIOLOGICAL BASIS OF MORPHOLOGICAL POLARITY IN REGENERATION. II.

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I. INTRODUCTION.

The preceding papers have shown that the phenomenon of regeneration of shoots in *Bryophyllum calycinum* is to be treated as a problem of nutrition and growth, since equal masses of sister leaves, when cut off from the plant, produce under equal conditions and in equal time approximately equal masses of shoots though the number of shoots may differ considerably;[1] and since stems split longitudinally, each retaining one sister leaf, produce also approximately equal masses of shoots in equal times and under equal conditions.[2] When the masses of two sets of sister leaves are made unequal, the mass of shoots produced by the leaf or by the stem varies approximately in proportion to the mass of the leaves. This law holds only when the leaves are exposed to light and it seems difficult to interpret it in any other way except that it is primarily the mass of the material produced in or sent out by the leaf which determines the mass of shoots produced in these cases.

Phenomena of regeneration show a second characteristic which we may designate briefly as that of morphological polarity, whereby we mean that an isolated piece of a plant or animal will as a rule produce different organs at opposite ends. If regeneration is a phenomenon of nutrition or chemical mass action, it will also become necessary to account for this polarity in terms of nutrition or mass action. We have commenced an investigation of the physiological basis of polarity and have published already some results which have

[1] Loeb, J., *Bot. Gaz.*, 1918, lxv, 150.

[2] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 81.

also shown a dominating influence of the mass of the leaf.[3] Thus in *Bryophyllum calycinum* a leaf at the apex of a piece of stem will inhibit the formation of shoots in the basal part of the stem and this inhibitory influence disappears when the mass of the leaf is diminished below a certain limit. This inhibitory influence of the leaf upon shoot formation follows the path of the conducting vessels from the leaf into the stem. Hence a leaf favors shoot formation at the apex of a piece of stem and inhibits shoot formation at the basal parts of the stem, and both phenomena are a function of the mass of the leaf.

This influence of the leaf exists in the normal plant as well as in the regenerating plant; *i.e.*, in a piece cut out from the plant. The normal stem of *Bryophyllum calycinum* has two dormant buds in each node capable of growing into shoots, though they never do so in a normal plant. This is partly due to the influence of the apical leaves and this influence is responsible for the fact that the normal stem of *Bryophyllum* is unbranched. We say partly due to the leaves since the growing region at the apex acts in a way similar to a leaf.[3]

We intend to show in this paper that the leaf not only influences shoot formation but also root formation.

II. Influence of the Mass of an Apical Leaf on the Mass of Air Roots Formed.

Pieces containing a number of nodes were cut out from healthy plants of *Bryophyllum calycinum*. All the leaves except the two apical ones were removed and the stems were split longitudinally so that each half of stem possessed one apical leaf. One leaf was usually left intact; the mass of the other leaf was reduced by cutting away part of the leaf. The two sets of half stems were suspended horizontally in a moist aquarium with the cut surface above (Figs. 1 and 2). On the lower side of the stems roots soon formed, first generally at the second node behind the leaf, and later at the base and at other nodes. At the base the formation of roots was most abundant. Still later roots were also formed in the internodes, especially in the region of geotropic curvature.

[3] Loeb, J., *Science*, 1917, xlvi, 547; *J. Gen. Physiol.*, 1918–19, i, 337.

FIG. 1. Stems split longitudinally and suspended horizontally, each half stem having one leaf at the apex; stem on left, whole leaf; stem on right, leaf reduced in size. Considerably more roots are produced in the half stems with whole leaf on left than on right where the mass of leaf is diminished. Geotropic curvature is also greater where the mass of leaf is greater. Duration of experiment 36 days.

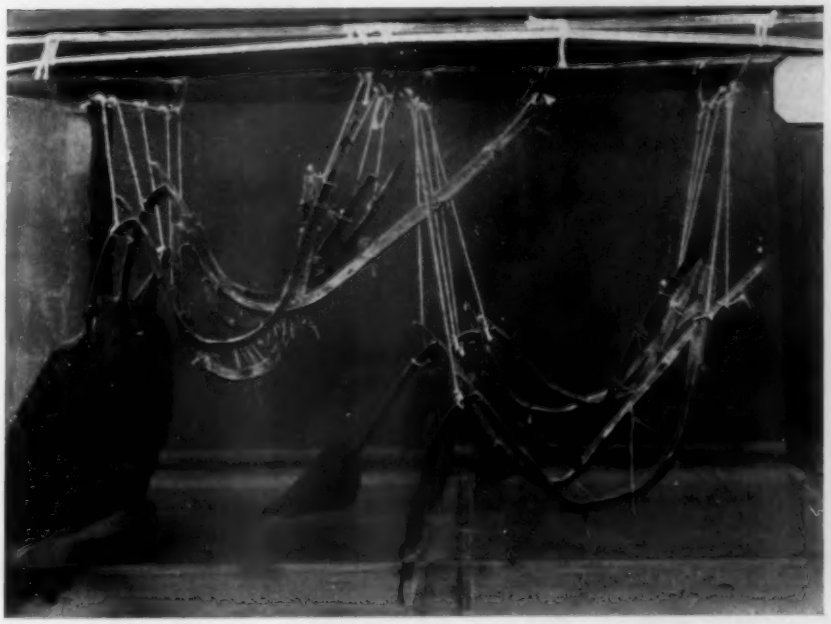

FIG. 2. Repetition of experiment in Fig. 1. Duration of experiment 25 days.

It was expected and found that the larger the mass of apical sister leaves the greater the mass of roots produced in equal time and under equal conditions. Figs. 1 and 2 are typical experiments of this kind. It was not possible, however, to prove that the two masses of roots formed (in about 5 weeks) are always proportional to the masses of leaves. This may be due to two sources of error inherent in experiments on root formation. First, the roots we are dealing with are air roots which usually dry out and fall off after a short time, while new air roots begin to form. This alone would make it almost impossible to prove an exact proportionality between mass of leaf and of root production even if it existed. The second difficulty lies in the fact of the small mass of each root, which makes the error in cutting off and in ascertaining the mass of the roots rather large. The fresh as well as the dry weight of the roots was determined in each case. The data given in Table I, however, leave no doubt that the mass of roots increases when the mass of leaves increases, though no mathematical expression of the relation can be deduced, for the reasons stated. When the half stem has no leaf the root formation is greatly retarded and diminished if not suppressed, as is shown in Fig. 3. One set of half stems had an apical leaf, while the other set had no leaf. The stems were lying with their cut surface on moist filter paper. The stems without a leaf formed practically no roots though they formed apical shoots. The other set of half stems with an apical leaf formed an abundant supply of roots but the shoot formation was suppressed by the apical leaf. The drawing was made 26 days after the beginning of the experiment.

The writer has repeatedly called attention to the fact that not only the root formation but also the geotropic curvature of the stems increases with the mass of the apical leaves,[4] and this fact was confirmed in the new experiments. The stems in Figs. 1 and 2 were originally perfectly straight, and the curvature noticeable in the photographs is due to the growth in length of a certain layer of cells of the cortex. These experiments show, in the opinion of the writer, that the phenomenon of geotropic curvature is also a phenomenon of

[4] Loeb, J., *Bot. Gaz.*, 1917, lxiii, 25; *Ann. Inst. Pasteur*, 1918, xxxii, 1.

TABLE I.

Showing that the Mass of Air Roots in a Stem Diminishes with the Mass of the Apical Leaf. The Stems were Split Longitudinally, Each Half Possessing One Sister Leaf at the Apex. In One Set the Leaf Was Left Intact, in the Other It Was Reduced in Size by Cutting Off Part of the Leaf. The Stems with Reduced Leaf Invariably Formed a Small Mass of Roots.

No. of experiment.	Date and duration of experiment.			Weight of leaves.	Weight of air roots on stem.	Approximate ratio of weight.	
						Of leaves.	Of roots.
				gm.	gm.		
1	Jan. 15 to Feb. 21, 1918.	6 whole leaves,	*fresh*	19.03	0.054	6.7	6.7
		6 sister leaves reduced in size,	*fresh*	2.85	0.008		
2	Oct. 29 to Dec. 9, 1918.	5 whole leaves,	*fresh*	51.1	0.077	3	3
			dry	2.627	0.016		
		5 sister leaves reduced in size,	*fresh*	16.33	0.025		
			dry	0.901	0.005		
3	Jan. 9 to Feb. 17, 1919.	5 whole leaves,	*fresh*	11.65	0.043	4	2
			dry	0.526	0.006		
		5 sister leaves reduced in size,	*fresh*	2.935	0.022		
			dry	0.132	0.003		
4	Jan. 22 to Mar. 6, 1919.	6 whole leaves,	*fresh*	34.462	0.128	3.3	6.1
			dry	1.378	0.037		
		6 sister leaves reduced in size,	*fresh*	10.032	0.026		
			dry	0.416	0.006		
5	Jan. 31 to Mar. 3, 1919.	5 whole leaves,	*fresh*	32.8	0.044	3	2
			dry	1.504	0.009		
		5 sister leaves reduced in size,	*fresh*	8.625	0.026		
			dry	0.485	0.005		
6	Oct. 27 to Dec. 9, 1918.	5 whole leaves,	*fresh*	14.4	0.423	11.6	6.4
			dry	1.36	0.109		
		5 sister leaves reduced in size,	*fresh*	1.57	0.900		
			dry	0.117	0.017		

nutrition and growth and that Haberlandt's hypothesis of an otolith function of starch granules as the cause of geotropic curvature is neither needed nor warranted. The hypothesis of geotropic "stimulation" had better be replaced by the application of the law of mass action.[5]

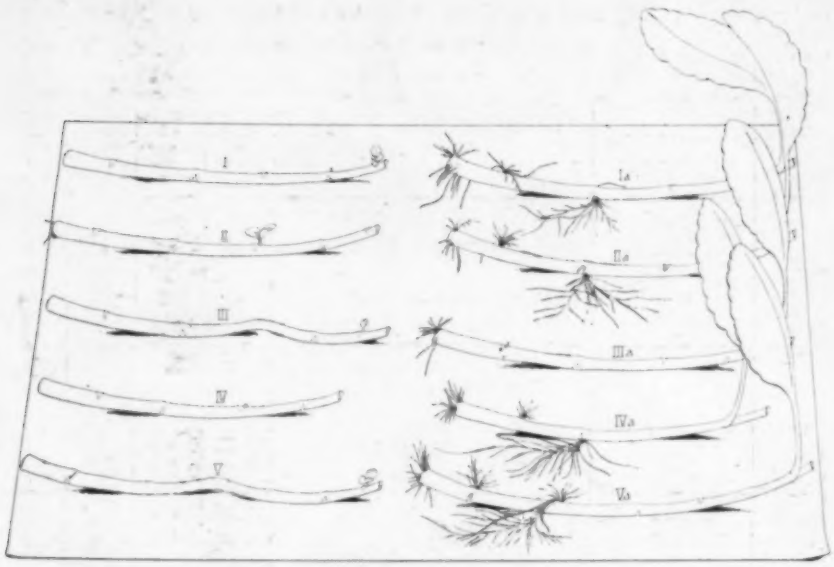

FIG. 3. I and I *a*, II and II *a*, etc., halves of the same stem split longitudinally and put with their cut side on moist filter paper. Half stems I *a*, II *a*, etc., on right, having each a leaf at apex, form considerable masses of roots on stem, while half stems I, II, etc., on left, having no apical leaf, form practically no roots in the same time. Duration of experiment 26 days.

III. The Influence of Light Upon Root Formation in the Stem.

Six stems were split longitudinally into two pieces, each with an apical leaf as described. One-half of each stem was suspended horizontally, with the cut surface above, in a dark aquarium, the other in an aquarium exposed to light. The temperature was the same in both aquaria. After 22 days photographs (Figs. 4 and 5) were taken. The stems exposed to the light (Fig. 4) had formed a

[5] Loeb, J., Forced movements, tropisms, and animal conduct. Monographs on Experimental Biology, Philadelphia, 1918, 120.

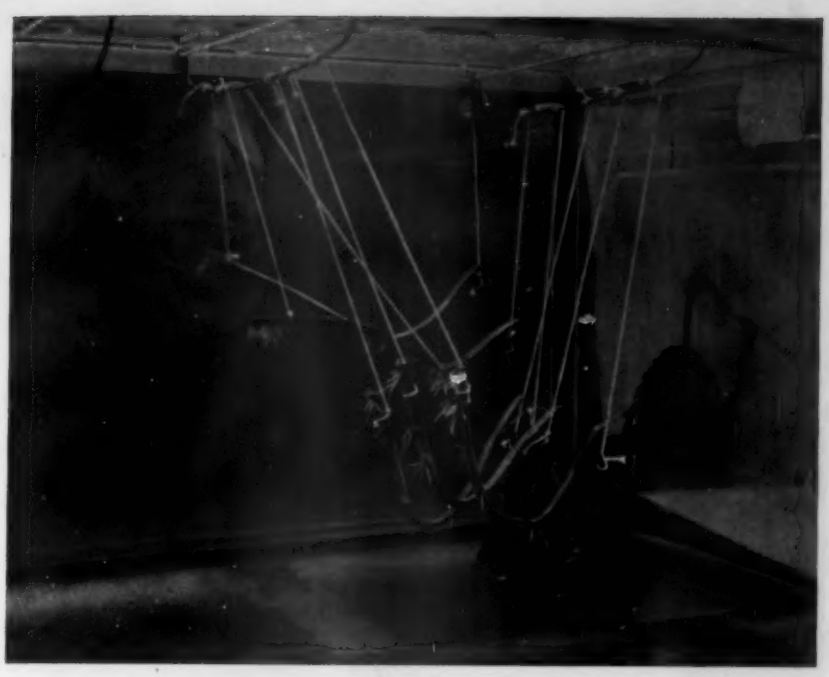

FIG. 4.

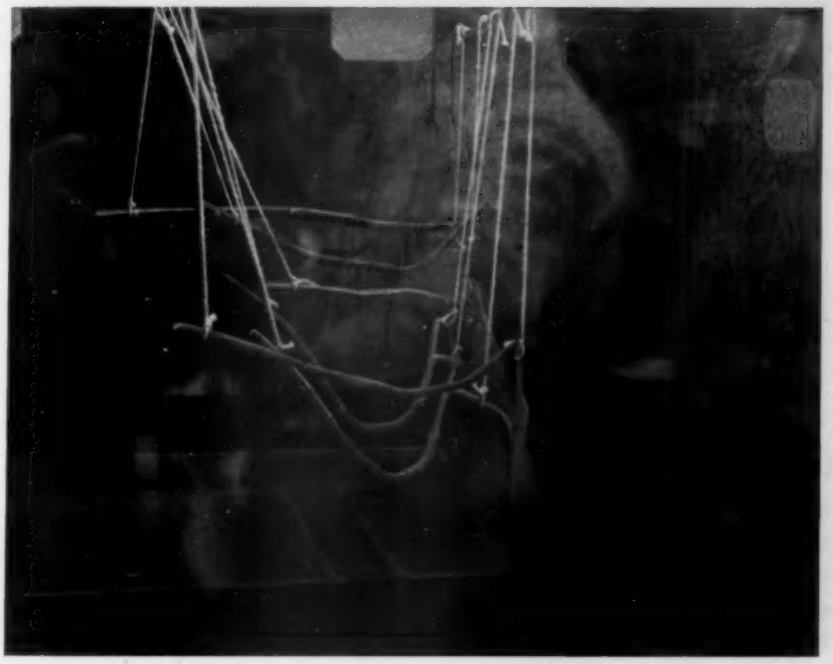

FIG. 5.

FIGS. 4 and 5. Six stems split longitudinally each half stem possessing one apical leaf. Half stems in Fig. 5, kept in dark, produce practically no roots while the half stems in Fig. 4, exposed to light, form an abundance of air roots. Duration of experiment 22 days.

considerable mass of air roots while the stems kept in the dark (Fig. 5) had formed practically no roots. In the dark the leaves have a tendency to fall off but we are not ready to attribute the non-appearance of roots on the stems kept in the dark purely to the interruption of the sap flow from leaf to stem. It is probably also partly due to the interruption of the process of assimilation in the leaf. This is suggested by the fact that we get the same inhibition or

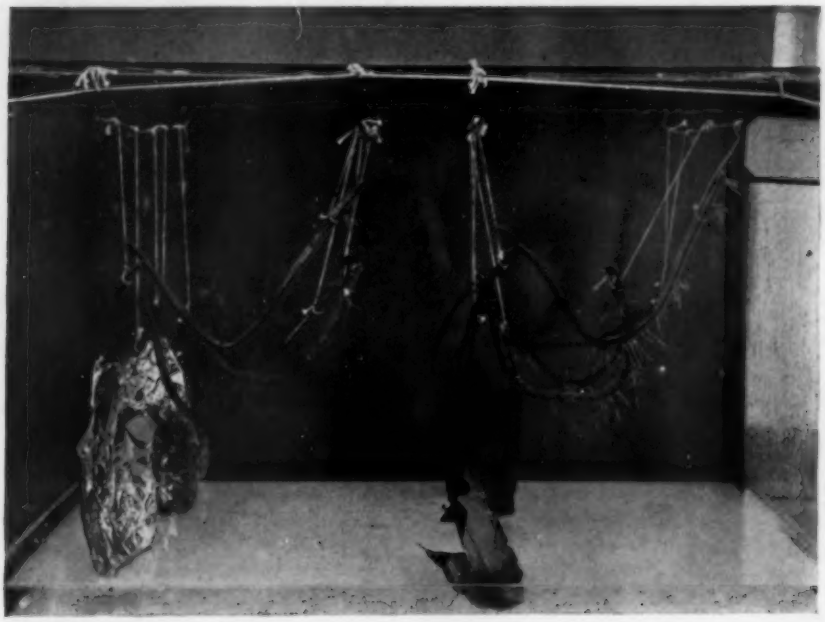

FIG. 6. Stems split longitudinally, each half stem having one apical leaf. Stems on left, having their leaves covered with tin-foil, produce only few roots, while stems on right, having their leaves exposed to light, produce in the same time a considerable mass of air roots. Duration of experiment 27 days.

suppression of root formation when we cover the leaf with tin foil in which case the tendency of the leaf to fall off is less striking. Figs. 6 and 7 are illustrations of such an experiment after 27 days. There was less difference in the geotropic curvature of stems with leaves in the light and in the dark than in the mass of roots produced. This may be due to the fact that the geotropic curvature occurs at an

earlier date than the root formation; hence the substances existing in the leaf at the time of darkening may suffice for bringing about the geotropic curvature but will not suffice for bringing about ample root formation.

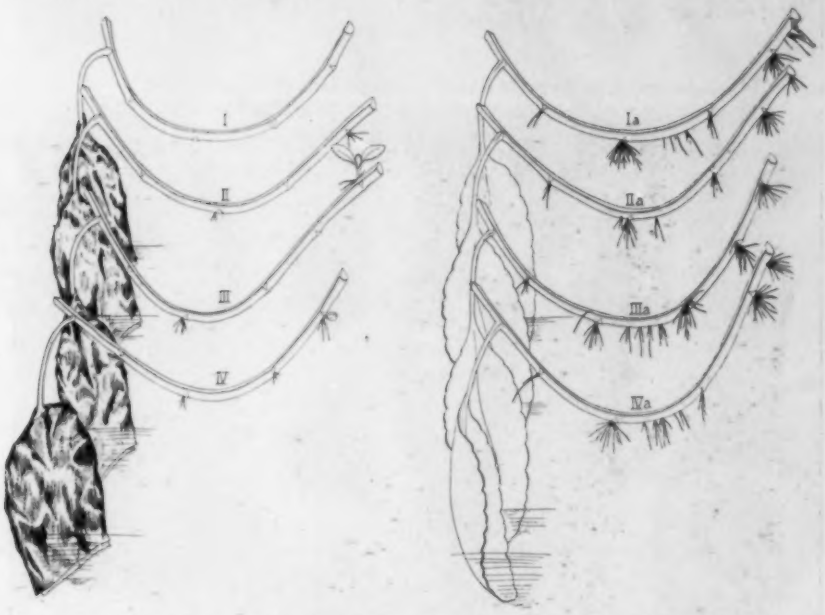

FIG. 7. Drawing of the same stems as in Fig. 6.

IV. Effect of Gravity on Root Formation.

Figs. 8 and 9 are photographs of whole stems suspended horizontally, each stem having an apical leaf on the upper side. All these stems were originally perfectly straight. The reader will notice that all the stems have an abundant supply of roots but these roots are all on the lower side of the stem with the exception of the extreme basal end. The mass of the leaves was reduced in one set of stems (Fig. 8) and the mass of roots was less in this set than in the other (Fig. 9), where the mass of the leaves was larger, thus confirming our statement at the beginning of this paper. The stems with a larger mass of leaves have undergone also considerably greater curvature than the stems with a smaller mass of leaves.

There is another connection between root formation and geotropic curvature. We have shown in a preceding paper[4] that the curvature is due to the fact that the under side of the cortex of a stem suspended

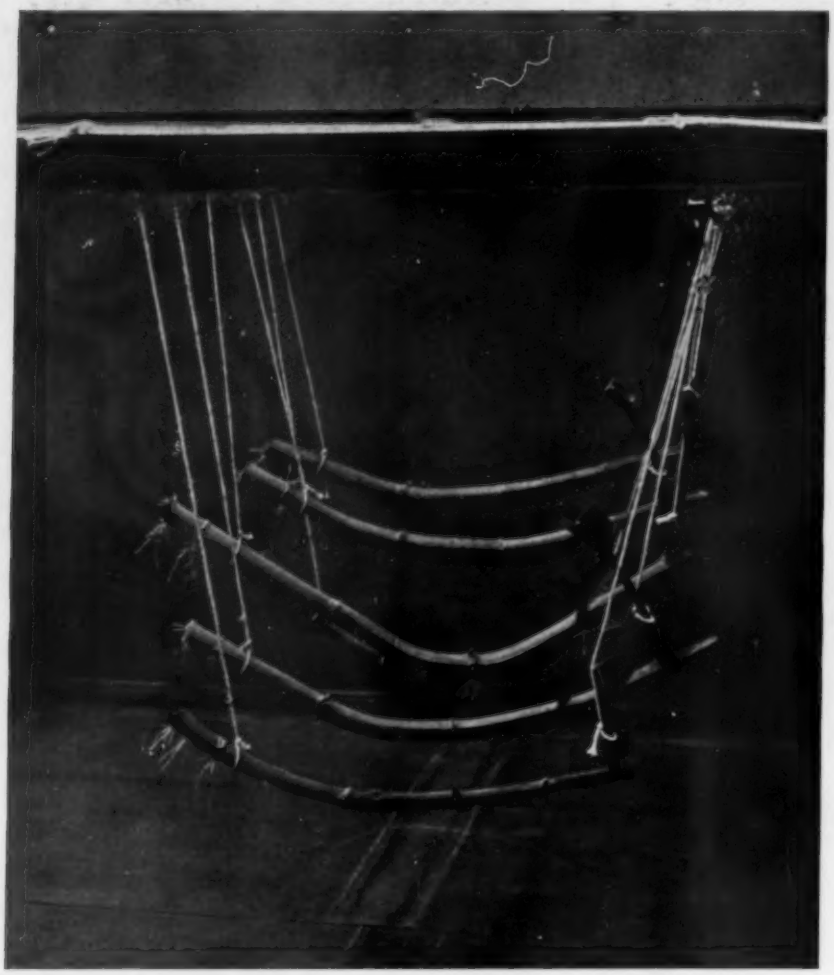

FIG. 8.

horizontally continues to grow in length while the upper side does not do so to any appreciable degree. The photographs (Figs. 8 and 9) show that if we disregard the basal end of the stem, the roots develop

FIG. 9.

FIGS. 8 and 9. Whole stems with leaf at apex on upper side. Stems in Fig. 8 with leaves reduced in mass produce fewer roots than stems in Fig. 9 with whole leaves. In both cases roots are formed on the under but not on the upper side of stems, except at the extreme cut end. The geotropic curvature is greater in stems with whole leaves (Fig. 9) than in stems with reduced leaf (Fig. 8). Duration of experiment 36 days.

only on the basal side of the stem. The fact that no roots grow on the upper side of the stem cannot, however, be dismissed with the mere statement of an influence of gravity in this case. Fig. 4 shows that an abundance of roots will be formed on the upper side of a horizontal stem when the lower side is cut off. Why then will no roots develop on the upper side of an intact stem suspended hori-

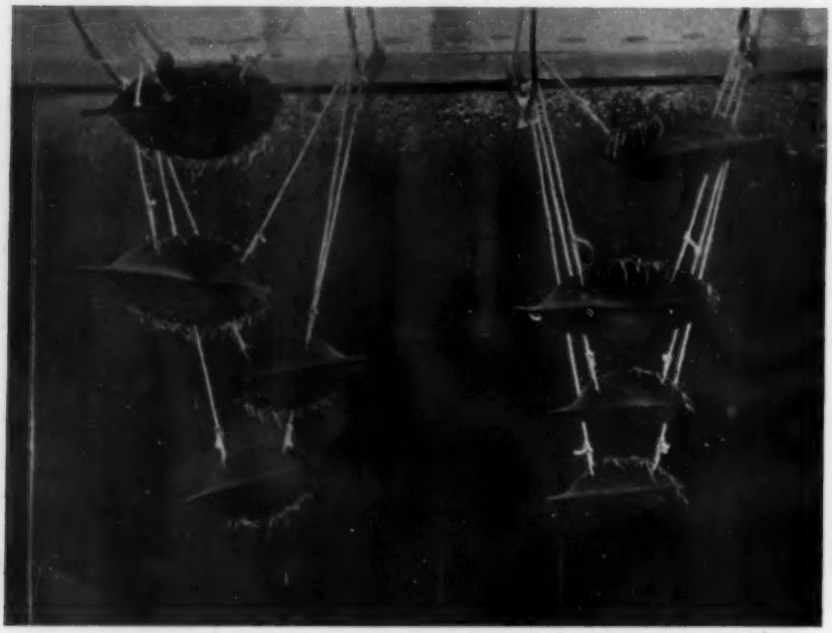

FIG. 10. Leaves suspended sidewise in air. On left side of photograph more roots are formed on the lower than on the upper side of leaf; in the leaves on the right side of the photograph the lower edge is cut off. In this case the upper edge of the leaves produces the roots and shoots which otherwise would have developed below. Duration of experiment 25 days.

zontally? The reason is that the roots grow more rapidly on the lower side of a stem suspended horizontally and that on account of this more rapid growth of the roots on the under side the growth of roots on the upper side of the stem is inhibited. When we remove the under side of a stem this inhibitory effect disappears and roots will grow from the upper side of the stem.

The correctness of this reasoning (which is of importance for the theory of polarity) can be demonstrated by experiments on the production of roots in isolated leaves. When isolated leaves of *Bryophyllum calycinum* are suspended sidewise in a moist atmosphere, an abundance of roots is formed on the lower side of these leaves and none or fewer on the upper side (see left half of Fig. 10). This is due to the fact that roots commence to develop a little earlier in the notches on the under than on the upper side of such a leaf and that they grow in general a little more rapidly on the lower than on the upper side. The more rapidly growing elements in a leaf inhibit the growth of the more slowly growing elements of the same kind.[1] The correctness of this interpretation is demonstrated on the right half of the photograph. The four leaves on the right side of Fig. 10 are the sister leaves of the four leaves on the left. The lower edge of the leaves on the right side was cut off, thus preventing the growth of roots on the lower side, since roots only grow out from preformed buds in the notches of a leaf. In this case the roots on the upper side grow out more abundantly. The photograph was taken on the 25th day of the experiment. Figs. 11 and 12 demonstrate another experiment of the same type. Fig. 11 shows that in the intact leaves on the left half of the drawing, the roots grow out sooner on the lower than on the upper side. The drawing was made after 8 days. In the sister leaves on the right side of the drawing, the lower edge of each leaf was cut off, thus preventing root formation on the lower side of the leaves. Practically no roots had formed on the upper side of the leaves. Fig. 12 is a drawing of the same leaves 25 days later. In the leaves on the right side, where the notches on the under side were cut off, the growth of roots and shoots on the upper side is much more abundant than it is on the upper side of the sister leaves on the left side of Fig. 12, where the growth on the upper side was retarded or inhibited by the more liberal growth on the under side of the leaves. The mass of shoots in each pair of sister leaves was practically in proportion to the mass of the leaves.

A striking demonstration of the principle that acceleration of root (and shoot) formation in one part of a leaf inhibits the root (and shoot) formation in the other part of the leaf is offered in Figs. 13 and 14. The four leaves in Fig. 13 were put with their apices into water which

accelerates root formation in the notches. An enormous growth of roots occurred in the watered notches. This suppressed the growth of roots in the other notches of the leaf and caused the air roots already formed there to shrink and dry up soon after they were formed.

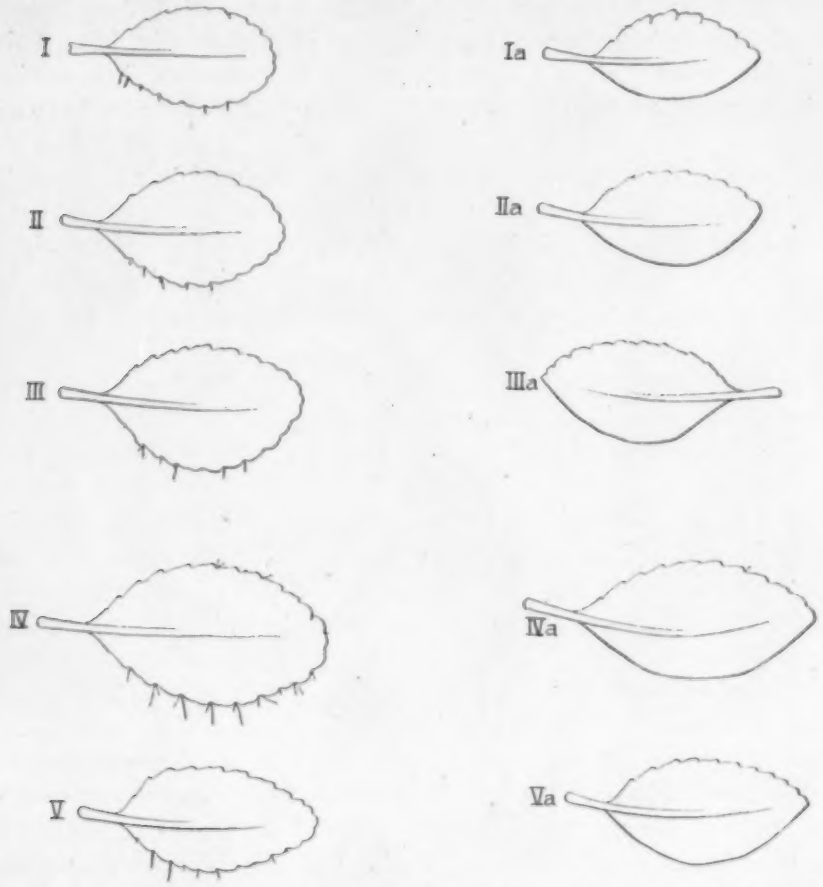

FIG. 11. Similar experiment as in Fig. 10, showing that roots form first at lower edge of leaves suspended sidewise. Drawn on 8th day of experiment.

The drawing was made on the 17th day. Fig. 14 is a drawing of the sister leaves, made also after 17 days. These sister leaves were not dipped in water, but were suspended in moist air. Hence no special acceleration of root formation took place in any part of the leaf.

As a consequence we have an abundant supply of roots in practically every notch of the leaf. In the upper lateral leaves which are suspended sidewise the growth of roots is again more abundant in

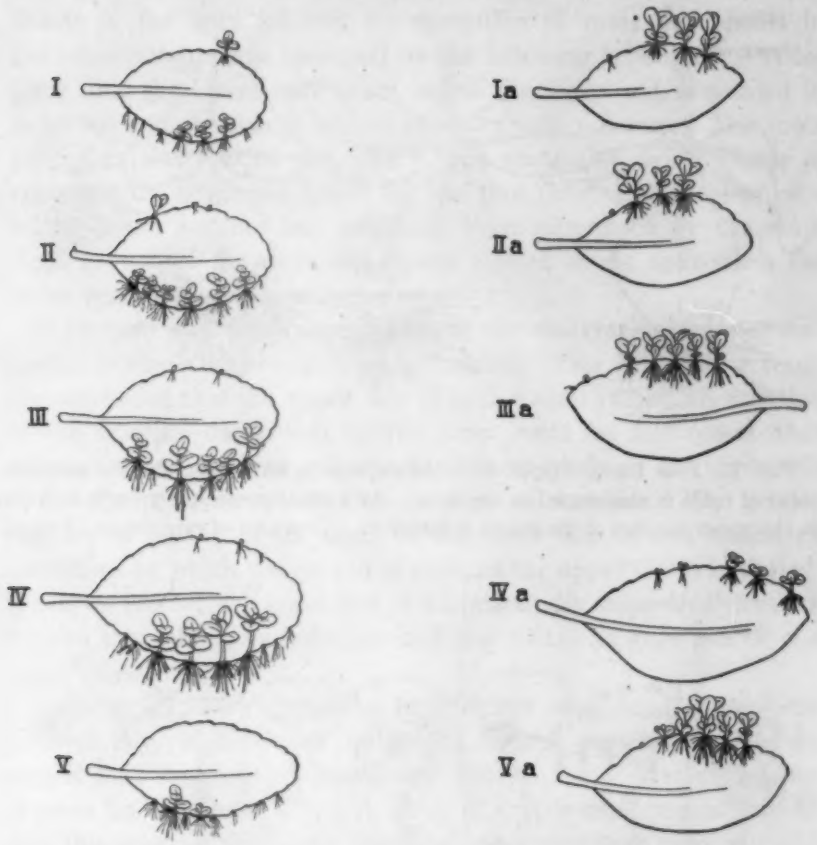

FIG. 12. The same leaves as in Fig. 11, but 25 days later, showing that the more rapid development of roots on the lower side of Leaves I to V retards or inhibits the development of roots on the upper side of the leaves. In Leaves I *a* to V *a*, where the lower edge is cut off, the development of roots and shoots on the upper side is not restricted.

the notches on the lower side, while no such influence of gravity is noticeable in the lower central leaves. This difference has been discussed in a previous paper and the influence of gravity on the

development of roots in the leaves suspended sidewise was explained as being due to a greater collection either of water or of solutes or of both in the notches on the lower side.[1] This influence of gravity on

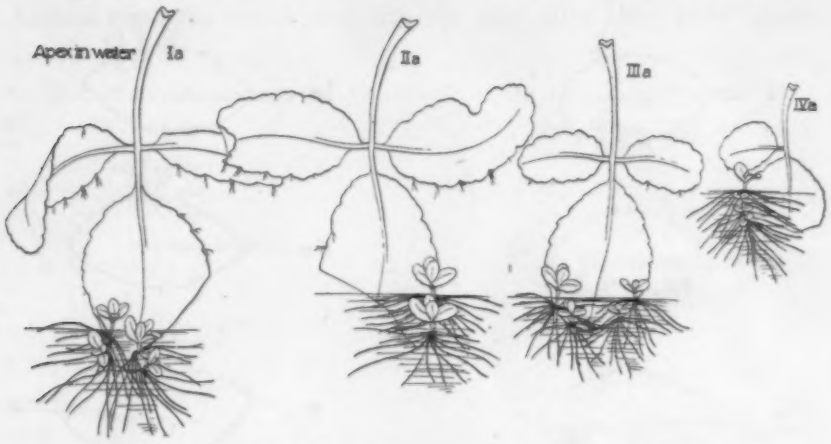

FIG. 13. Four leaves dipped with their apices in water whereby the development of roots is accelerated in the apex. As a consequence the growth of roots in the upper notches of the leaves is inhibited. Duration of experiment 17 days.

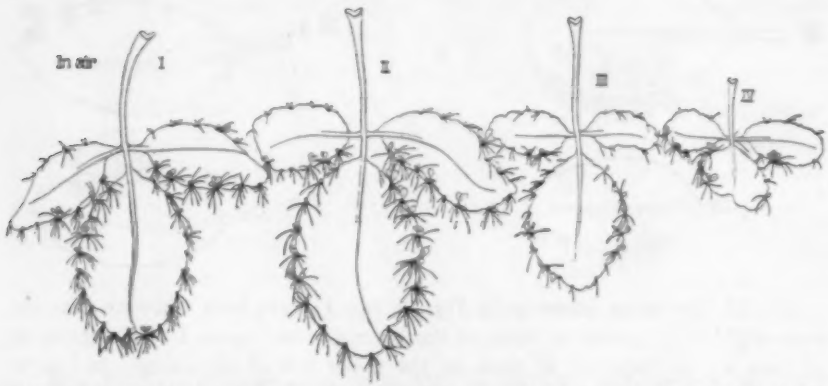

FIG. 14. Sister leaves of those in Fig. 13 suspended in moist air. Most of the notches have roots since the growth is nowhere sufficiently accelerated to retard it in the rest of the notches. Duration of experiment 17 days.

the collection of water can show mainly in the more fleshy parts of the leaf, *i.e.* in the middle, but not in the thin tissues of the apex of the leaf.

When we dip isolated leaves with their apices into water an enormous root and shoot formation will occur in the notches of the apex but nowhere else. The proof that the rapid growth of roots and shoots in the apex inhibits the formation of roots and shoots in the other notches was furnished by the following experiment. After some time the leaves were taken out of the water and suspended in moist air and the shoots formed in water were removed. The roots formed in water dried out. Very soon roots and shoots began to appear in the upper notches of the leaf thus proving that the growth in the upper notches had originally been suppressed by the more rapid growth of the roots and shoots formed at the apex when the latter was dipping into water.

If we turn from these experiments to the observations on root formation in whole stems suspended horizontally (Figs. 8 and 9), we reach the conclusion that the upper side of such a stem (with the exception of the extreme basal end) is free from roots for the reason that water or solutes or both collect a little more freely on the lower side of the stem, thus favoring root formation on that side. The greater rapidity of growth of the roots on the lower side creates secondary conditions by which the growth of roots on the upper side is inhibited. When we remove the lower half of a horizontally suspended stem we remove this inhibitory influence and now roots can grow out on the upper side.

Since in all these cases the horizontally suspended stems bent geotropically, whereby the under side became convex, it might be argued that this convexity was the cause of the greater abundance of roots on the under side. A series of simple experiments showed that this was not the case. Pieces of stem were bent passively and tied in this position to sticks of wood (Fig. 15). They were suspended in such a way that they turned their concave side downwards. In all cases, without any exception, the roots developed on the lower sides of the stems which were now concave (Fig. 15, after 35 days). They also developed on the under side when the bending of the stem was prevented by cork rings and when the stem was enclosed in a glass tube, as in Fig. 16.

Reed and Halma[6] have shown that shoots grow out only on the upper side of horizontally suspended branches of citron trees. Our experiments published in a previous article show that shoots can grow out from the under side of a piece of stem of *Bryophyllum*, suspended horizontally, and that they invariably do so when the upper half

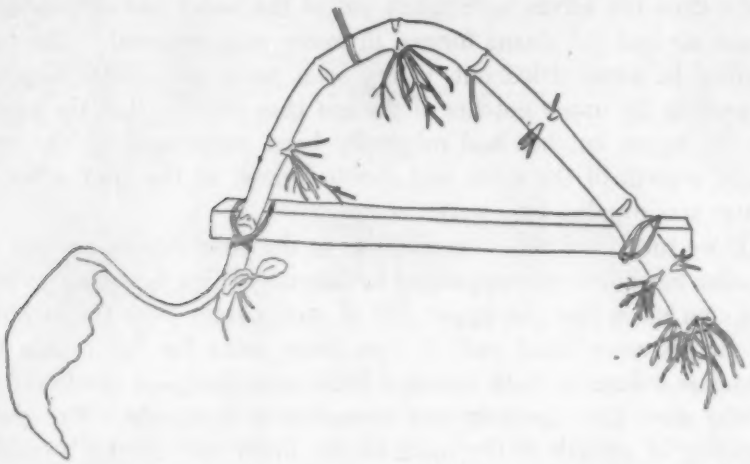

FIG. 15. Stem bent passively, concave side below. Roots form on lower, concave side. Duration of experiment 35 days.

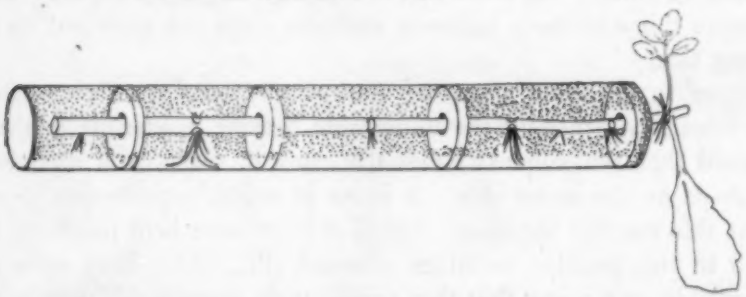

FIG. 16. Geotropic curvature prevented in stem. Roots are formed nevertheless on lower side. Duration of experiment 41 days.

of such a stem is removed. This suggests the possibility that if the upper half of a citron bough, suspended horizontally, is removed the shoots might grow out from the under side, and that in Reed and

[6] Reed, H. S., and Halma, F. F., *Univ. Cal. Pub., Agric. Sc.*, 1919, iv, 99.

Halma's experiment the formation of shoots on the under side of a piece of stem of citron when suspended horizontally was inhibited by the more rapid growth of the shoots on the upper side of such a piece of stem.

The writer has already reported an experiment[3] which proves directly that the growth of a shoot on the upper side of a stem of *Bryophyllum*, suspended horizontally, inhibits the growth of a shoot on the lower side of such a stem. When we isolate a piece of stem of *Bryophyllum* and remove all the leaves from such a piece the two buds situated in the most apical node will grow out into shoots. When we place a stem horizontally in such a way that the line connecting the two apical shoot buds is vertical, both buds will begin to grow out but the upper shoot will grow more rapidly than the lower one and in the majority of cases the lower shoot will soon stop growing, while the upper shoot continues to grow. When we remove the upper bud, this inhibitory effect on the lower bud disappears and the shoot on the lower side will grow out as vigorously as the upper shoot would have done if its bud had not been removed.

Our experiments show also that the path for the substances favoring root formation is not identical with the path determining inhibition of shoot formation. Thus in Figs. 8 and 9 the inhibitory influences on shoot formation in the stem follow the conducting vessels on the upper half of the horizontally suspended stem, while the root formation is favored in the lower half of the stem.

V. The Preformation of Root Buds in the Axil of a Leaf.

We have seen that in the axil of each leaf of *Bryophyllum calycinum* there is one bud capable of growing out into a shoot, though this growth is inhibited in a healthy plant under normal conditions, and the mechanism of this inhibition determines also the polar character of regeneration as far as shoot formation is concerned. Roots can develop anywhere on the stem and this causes the appearance as if no preformation of root buds existed in the plant. Yet this appearance is deceptive since as a matter of fact in the axil of each leaf there is not only a preformed shoot bud but also a preformed root bud. Curiously enough, this root bud lies a little above (apically from) the shoot bud. Special experiments are required to demonstrate the

existence and activity of these root buds. The simplest method of doing this is represented in Fig. 17. Vigorous stems consisting of five or six nodes are deprived of all leaves except those at the base. These basal leaves should be large since, as we have seen, the mass of root formation increases *ceteris paribus* with the mass of the leaves. The stems are suspended vertically in moist air so that the basal cut end just dips into water. In about 3 or 4 days (at greenhouse temperature and with good illumination) shoots and roots begin to grow out simultaneously. Fig. 17 gives a picture of such an

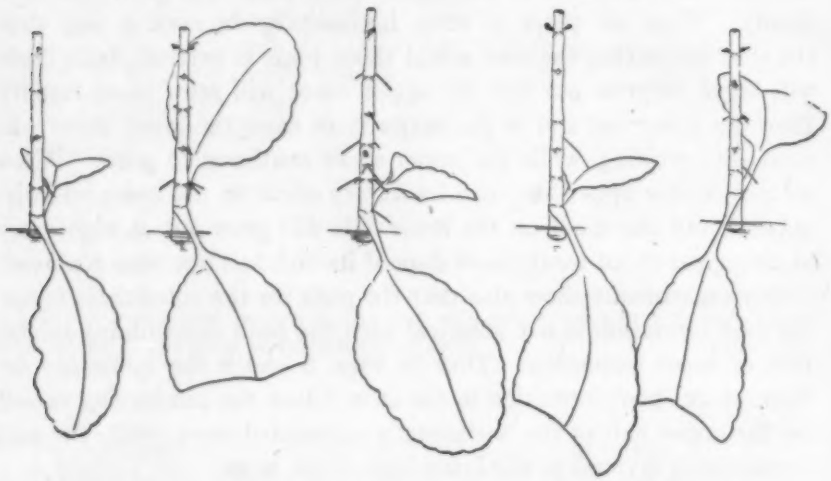

FIG. 17. Stems with two large leaves at base, the latter dipping in water, showing that roots form in many of the nodes situated apically from the leaf from buds situated above the shoot bud. Drawn after 6 days.

experiment after 6 days. The shoots are represented in thick black spots and the roots in double lines, thus imitating their natural appearance since the buds are at first dark red and the roots are, of course, white. It is obvious that each root takes its origin above (apically from) the bud.

About a week or 8 days after the preformed root buds have grown out from the stem, the formation of roots at the base near the cut end begins (Fig. 18). Under the conditions of our experiment they touched the water and began to grow very rapidly. As soon as

this happened, the air roots in the upper part of the stem began to dry out and were not replaced by new roots. The inference must be drawn that the luxurious growth of the roots at the basal end of

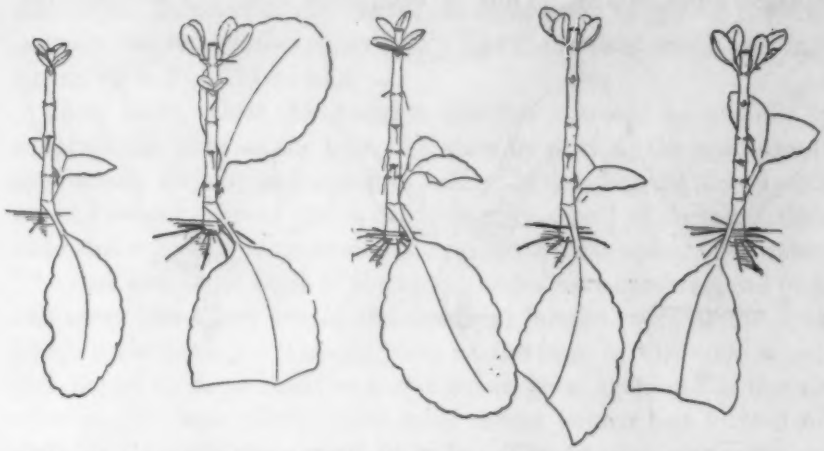

FIG. 18. The same stems as in Fig. 17, 8 days later. The basal roots in water have now commenced to develop and as a consequence the air roots, located higher up and formed before, commence to dry out and fall off.

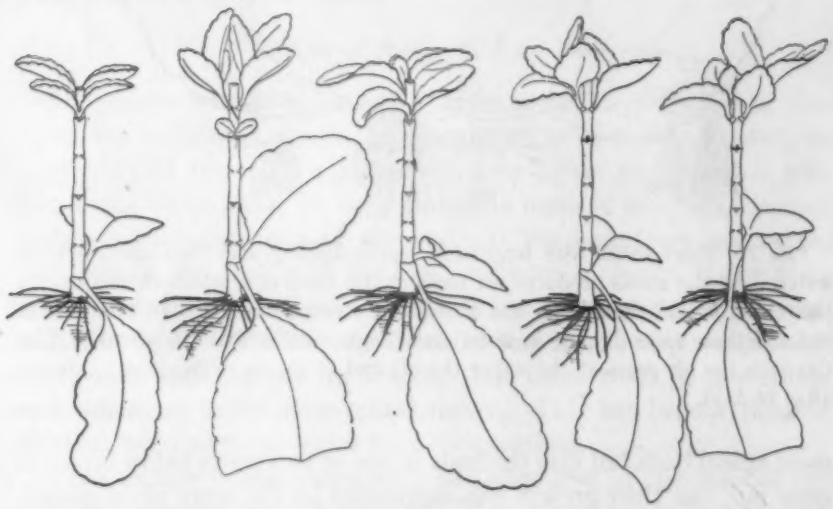

FIG. 19. The same stems as in Fig. 18 but 9 days later. The air roots have practically disappeared. The basal roots in water are developing rapidly.

the stem inhibited the further growth of the roots in the more apical nodes of the stem; in the same way as the more rapid growth of the most apical buds of a stem suppresses in general the growth of buds situated more basally. Thus in each stem (Fig. 17) not only the

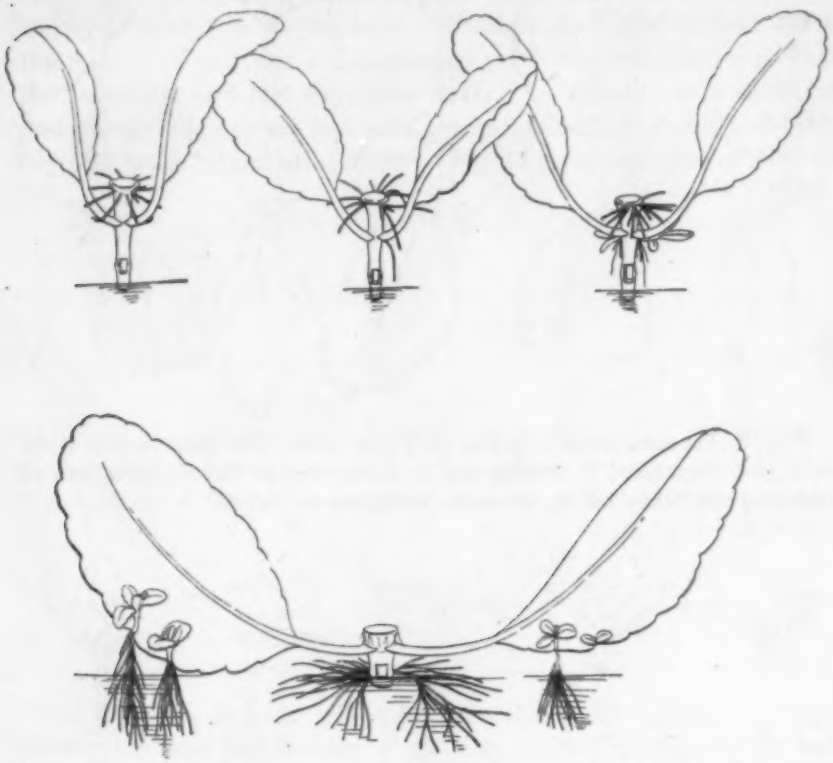

FIG. 20. Short stems with large basal leaves dipping with their apical ends in water. All the stems produced air roots at the basal end, which in this case is above and in air. The lower one of the four stems formed roots at its apical end and these roots dipping in water grew more rapidly than the air roots. In this case the air roots at the upper (basal) end of the stem dried out. Drawn after 26 days.

most apical buds but also the buds in one or two nodes below began to grow out, but their growth was suppressed by the more rapid growth of the most apical bud (Fig. 18). Fig. 19 is a drawing of the same stems 9 days later. Hardly any trace of the air roots in the upper

part of the stem is now left, while the basal roots have developed. Only at the base of the apical shoots roots may continue to grow.

The suppression of the growth of the more apical roots at the time when the roots at the base of the stem begin to grow is the analogue of the suppression of roots in a leaf the apex of which dips into water, as in Figs. 13 and 14.

These ideas raised the question whether it would be possible to suppress the roots at the base of a stem by putting the apical roots into water, thereby accelerating their growth. Fig. 20 shows such an experiment. Short pieces of stems with a pair of leaves at their base and one node in front were dipped with their apices into water. The root and shoot buds of the apical nodes were removed and only one stem (the lower one in the drawing) formed roots at the apex which grew rapidly. The air roots at the base of this stem which had begun to form dried out and never grew again while the air roots at the base of the three other stems (which had formed no roots at the apex) continued to grow. The drawing was made on the 26th day of the experiment. It is, however, not very easy to cause abundant root formation at the apex and hence this experiment will have to be repeated.

VI. The Rôle of Nodes in Root Formation.

The reader will have noticed that in order to demonstrate that roots can be formed also in the apical regions of a piece of stem we used isolated pieces of a stem with two leaves at the basal end. The basal leaves must be large since the mass of new roots formed increases with the mass of the leaf. If the leaves are not at the base but in the middle of the leaf, or, more correctly, if the piece of stem basally from the leaf includes one or more nodes, the root formation in the stem apically from the leaf is generally suppressed. The nodes basally from the leaf act like basal roots in preventing root formation in the more apical region. This has been verified in different ways some of which may be described.

When we select long stems with one large leaf at the base of the stem, and suspend them horizontally in moist air, roots develop first at some of the nodes apically from the leaf, and later in abundance at the base of the stem near the cut end (Fig. 21). Though these

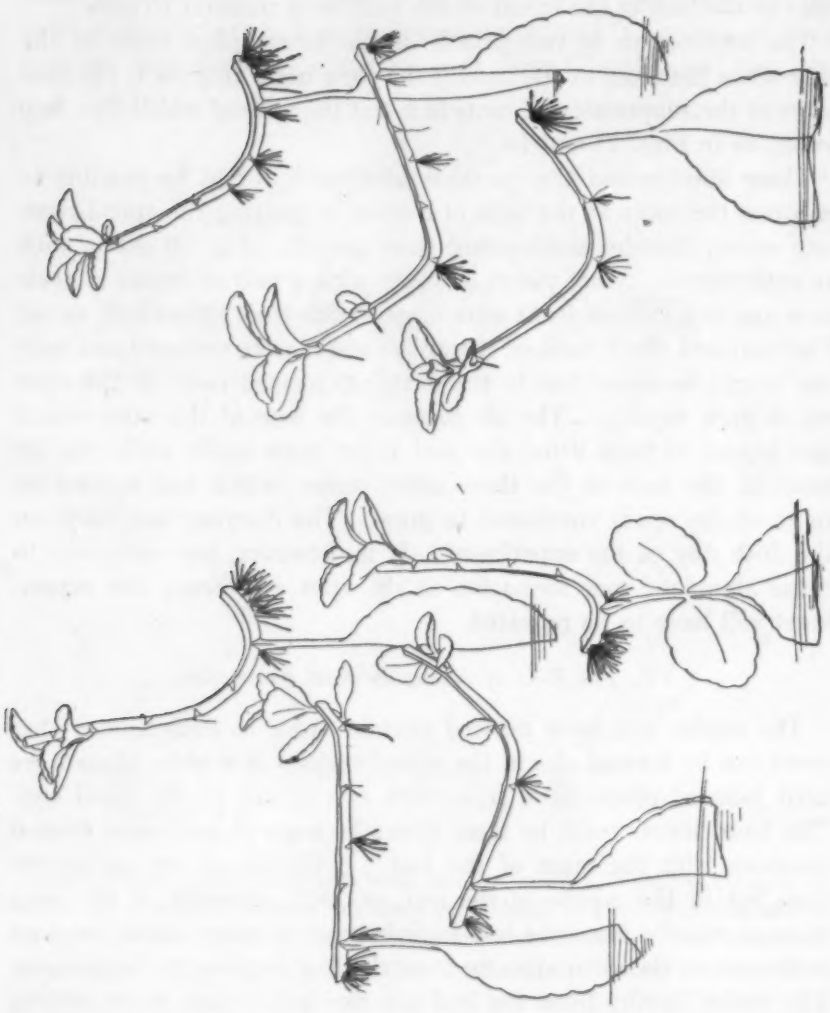

FIG. 21. Stems with leaf at base suspended in moist air. Roots developed first in some of the more apical nodes of the stem and persisted even after the basal roots had formed. The latter, being air roots, grew too slowly to inhibit the growth of the more apical roots. Drawn after 30 days.

latter roots will soon surpass in mass the apical roots, the apical roots will not wilt. The basal roots, when they develop in air instead of in water, do not grow rapidly enough to suppress the growth of the more apically situated roots. The drawing was made on the 30th day after the beginning of the experiment. The upper half of the stems had been cut off in this experiment. If, however, the leaf is not at the base but in the middle of the stem, as in Fig. 22, roots will

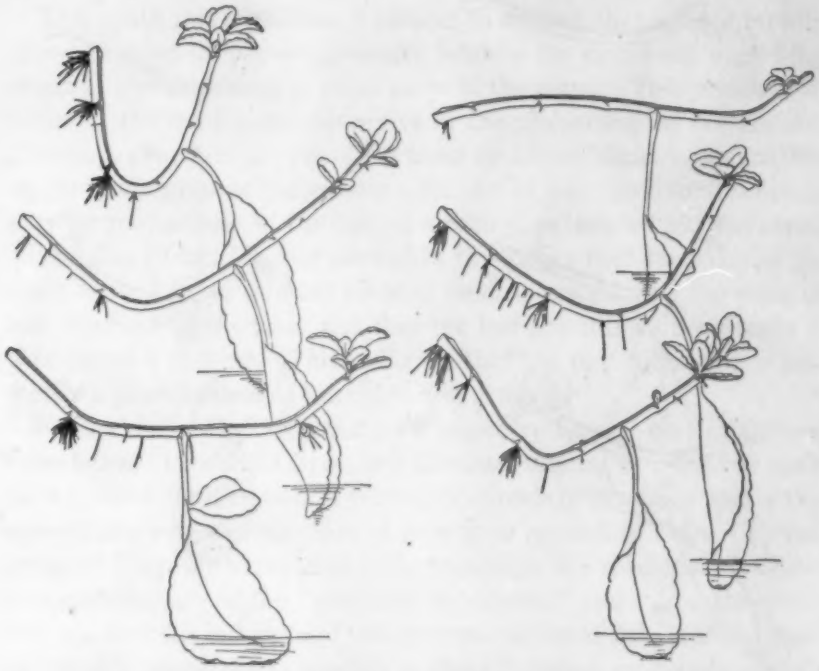

FIG. 22. Leaf in middle of stems suspended horizontally. Practically no roots develop in front of the leaf, the nodes basally from the leaf inhibiting the growth in the more apical nodes. Drawn after 24 days.

develop and persist only basally from the leaf. It is only necessary for this result that one node is preserved basally from the leaf, as in Fig. 23.

When we suspend a stem of *Bryophyllum calycinum*, possessing one leaf in the middle and on the under side of the stem, horizontally in moist air, the root formation will usually start first from the second

node and not from the first node behind the leaf.[7] The reason for this lies probably in the fact that the dormant root bud in the second node lies in the path of the descending sap from the leaf, while the root bud in the first node basally from the leaf lies above this path. We notice generally that in a piece of stem suspended horizontally,

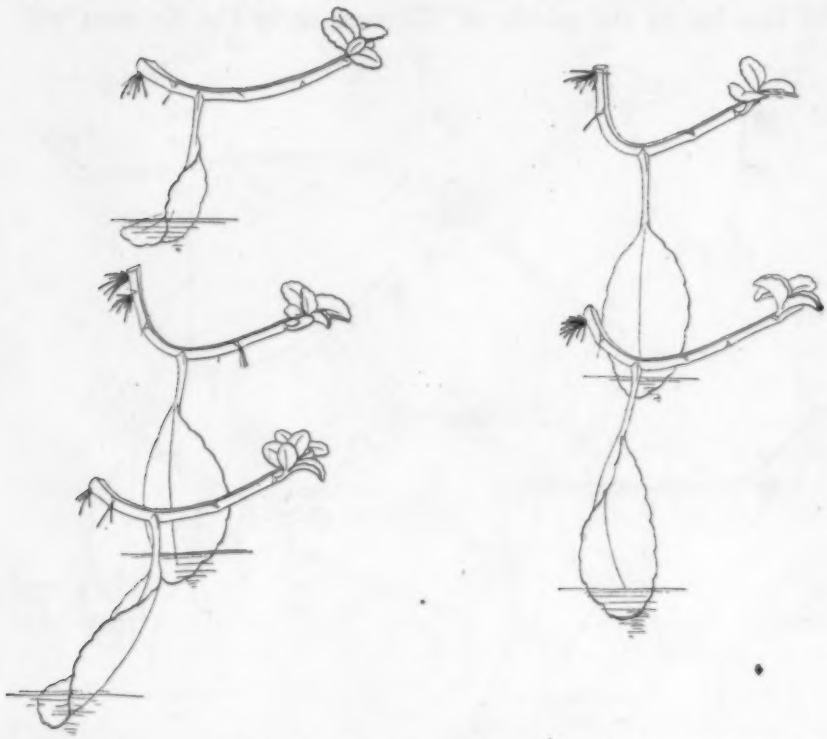

FIG. 23. Showing that one node behind the leaf suffices for this inhibition of the growth of roots in the buds situated apically from the leaf.

possessing one apical leaf on the under side and two nodes basally from the leaf, roots will grow out before they grow out in a similar piece of stem possessing only one node basally from the leaf.

[7] Loeb, J., *Bot. Gaz.*, 1917, lxiii, 25.

VII. General Remarks.

As far as the polar character of root formation is concerned, the experiments show that dormant buds for the development of air roots exist in the axil of each leaf (apically from the resting shoot buds) and that it is the rapid growth of the basal roots in the soil or in water which prevents these roots from growing out in the normal plant.

To explain this inhibition it suffices to assume that a more rapidly growing organ in a plant generally inhibits the more slowly growing organ of the same kind in other parts of the plant. This principle is, perhaps, the most generally active in the phenomena of correlation. The more abundant growth of the basal roots must find its explanation on the assumption of the greater collection of water and solutes necessary for root growth at the base of a normal or isolated piece of stem. This follows from the fact proved in this paper that the mass of air roots formed in an isolated piece of stem increases with the mass of leaf attached to the stem and that the leaf has this influence only if it is exposed to light. This indicates that the root formation is primarily a phenomenon of nutrition and growth.

Bayliss[8] has called attention to a paper by Errera[9] on "inhibitory stimulation" in which this author discussed the influence of the apex of fir trees on the direction of growth in secondary branches, and of the apex of roots on the direction of growth of secondary roots. Errera accepted Czapek's hypothesis of the formation of a specific antioxidase as a consequence of the "geotropic perception" and raised the question whether the influence of the apex on the lower ramifications does not consist essentially in sending to these branches, or in calling forth in these branches the formation of, a substance antagonistic to this antioxidase—a kind of antibody. This idea is, of course, very hypothetical, and the writer without being aware of Errera's suggestion had offered a different explanation of the same phenomenon.[7]

The assumption of the existence of specific inhibitory substances which the writer had used tentatively in two preceding papers,[3] and

[8] Bayliss, W. M., *Nature*, 1918, cii, 285.

[9] Errera, L., *Bull. Soc. Roy. bot. Belgique*, 1904, xlii, 27.

which was based on the experiments of geneticists, is not needed for the explanation of the results published in this paper.

In the next paper the writer intends to furnish direct proof for the fact that the isolation of a piece of a plant leads to a change in the flow and distribution of sap and that this change in the distribution determines the starting of growth of formerly dormant buds which is the essential feature in regeneration.

SUMMARY

1. The experiments show that the mass of air roots formed in a stem increases with the mass of the leaf attached to the stem, though it has not been possible to establish an exact mathematical relation between the two masses, owing to unavoidable sources of error.

2. Darkened leaves do not increase the mass of roots formed.

3. In stems suspended horizontally air roots appear on the lower side of the stem, with the exception of the cut end where they usually appear around the whole circumference of the stem. When the lower half of a stem suspended horizontally is cut off, roots are formed on the upper side. It is shown by experiments on leaves suspended horizontally that the more rapidly growing roots and shoots on the lower side inhibit the root and shoot formation in the upper half of such a leaf; and likewise the more rapid formation of roots on the lower side of a horizontally suspended stem seems to account for the inhibition of root formation on the upper side of such a stem. Likewise the more rapid growth of shoots on the upper side of a stem suspended horizontally is likely to inhibit the growth of shoots on the lower side.

4. Each leaf contains in its axil a preformed bud capable of giving rise to a root, which never grows out in the normal stem on account of the inhibitory influence of the normal roots at the base of the plant. These dormant root buds are situated above (apically from) the dormant shoot bud. The apical root buds can be caused to develop into air roots when a piece of stem is cut out from a plant from which the leaves except those in the basal node of the piece are removed. The larger these basal leaves the better the experiments succeed.

5. These apical air roots grow out in a few days, while the roots at the basal end of the stem (which in our experiments dip into water) grow out about a week later. As soon as the basal roots grow out in water they cause the air roots in the more apical region of the stem to dry out and to disappear.

6. In addition to the basal roots, basal nodes have also an inhibitory effect on the growth of the dormant root buds in the apical region of a stem. This is indicated by the fact that a stem with one pair of leaves near the base will form apical air roots more readily when no node is situated on the stem basally from the leaf than if there is a node basally from the leaf.

THE INFLUENCE OF ELECTROLYTES ON THE ELECTRIFICATION AND THE RATE OF DIFFUSION OF WATER THROUGH COLLODION MEMBRANES.

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I. INTRODUCTION.

The direct measurements of the osmotic pressure of sugar solutions by Morse[1] and his fellow workers as well as by Lord Berkeley and Hartley[2] show so close an approximation to the values calculated on the basis of van't Hoff's theory that it is difficult to doubt the correctness of this theory. As Fick[3] pointed out as early as 1866, we can, on the assumption of the validity of the kinetic theory, treat a solution as a mixture of two gases, the solvent and the solute. When the solution is separated from the pure solvent by a semipermeable membrane more molecules of the solvent will impinge in the unit of time on the unit area of the membrane on the side of the pure solvent than on the side of the solution and hence the solvent will diffuse to the solution until the partial pressure of the solvent on the solution side becomes so great, that the number of molecules of solvent impinging on the unit of membrane on the solution side becomes equal to the number of molecules impinging on the unit of membrane on the side of the pure solvent.

There are, however, phenomena which suggest that in addition to the gas pressure other forces may enter into the problem of diffusion of water through a membrane. Such phenomena are the cases of

[1] Morse, H. N., The osmotic pressure of aqueous solutions, *Carnegie Institution of Washington, Publication 198*, 1914.

[2] Earl of Berkeley, and Hartley, E. G. J., *Proc. Roy. Soc. London, Series A*, 1916, xcii, 477.

[3] Fick, A., Die medizinische Physik., Braunschweig, 1866, 2nd edition, 36; *Z. physik. Chem.*, 1890, v, 526.

so called negative osmosis in which the solvent moves from a solution of higher to one of lower molecular concentration and which have been described and studied by Dutrochet, Thomas Graham, Girard, Flusin, Bartell, Bernstein, Freundlich,[4] and others.

Girard, Bernstein, and Bartell have reached the conclusion that potential differences caused by the electrolyte play a part in these phenomena of abnormal osmosis, and our results are in agreement with this view. We cannot agree, however, with the view of Girard and of Freundlich that the H and OH ions play the decisive part in the electrical phenomena which lead to abnormal osmosis. This view of a privileged position of the H and OH ions in these phenomena rests on the famous experiments of Perrin[5] on electric endosmose. It had been known that when a galvanic current is sent through a porous diaphragm bounded on both sides by water the latter migrates to one of the two electrodes. No reason could be assigned for the direction of the migration, until Perrin made the remarkable discovery that when a current is sent through a colloidal diaphragm (gelatin, silk, etc.) the direction of motion of the water can be altered at will; addition of acid causes the water to move to the anode, addition of alkali to the cathode. This phenomenon was explained by Perrin on the assumption that the H or OH ions of the water are adsorbed by the surface of the diaphragm transferring their charges to the latter. A double layer is formed, the molecules of water adjacent to the diaphragm assuming the opposite electrical charge. However, Perrin noticed and pointed out a serious difficulty in this assumption, namely that no other monovalent ions except the H and OH ions are capable of such an apparent transfer of their charges to the diaphragm.

The writer is under the impression that we must discriminate between two kinds of diaphragms, namely those which consist of material

[4] Bernstein, J., Elektrobiologie, 1912. Bartell, F. E., *J. Am. Chem. Soc.*, 1914, xxxvi, 646. Bartell, F. E., and Hocker, C. D., *J. Am. Chem. Soc.*, 1916, xxxviii, 1029, 1036. Freundlich, H., *Kolloid-Z.*, 1916, xviii, 11. Girard, P., *Compt. rend. Acad.*, 1908, cxlvi, 927; 1909, cxlviii, 1047, 1186; 1910, cl, 1446; 1911, cliii, 401; La pression osmotique et le méchanisme de l'osmose, Publications de la Société de Chimie-physique, Paris, 1912. Flusin, G., *Ann. chim. et phys.*, 1908, xiii, 480.

[5] Perrin, J., Notice sur les titres et travaux scientifiques de M. Jean Perrin, Paris, 1918.

which is an amphoteric electrolyte (*e.g.* gelatin or silk) and those which consist of non-amphoteric material. Both types are not necessarily influenced in the same way by acids and alkalies. Amphoteric electrolytes form salts with acids as well as with alkalies and this salt formation reverses the influence of the diaphragm on the sign of electric endosmose. HCl transforms gelatin into gelatin chloride, and NaOH transforms it into sodium gelatinate. In the case of gelatin chloride the gelatin ion is positive and in the case of sodium gelatinate it is negative. We assume that it is primarily the sign of the gelatin ion which determines the sense of the electrification of water and that the OH and H ions play only a secondary part. This would explain why Perrin could find no other monovalent ion except H or OH which was able to reverse the electrification of the gelatin diaphragm, since only acids and alkalies can transform a gelatin cation into a gelatin anion, or *vice versa*, while neutral salts cannot produce such an effect.[6]

We are, therefore, of the opinion that the experiments on electrical endosmose through diaphragms of colloidal substances like gelatin, pig's bladder, silk, or any other amphoteric electrolyte, do not warrant the assumption of a specific influence or predominant position of the H and OH ions in the electrification of a membrane bounded on both sides by water, except in so far as acids and alkalies are the only substances which can reverse the sense of ionization of an amphoteric electrolyte.

Girard, in accepting Perrin's idea of a direct electrification of the wall by H and OH ions, and of the specific action of these two ions, tried to ascribe the phenomena of abnormal osmosis he observed with membranes of pig's bladder to the acid or alkaline reaction of the solution. Thus neutral solutions, like those of NaCl and Na_2SO_4, should be without any other except a purely osmotic effect and he publishes data apparently supporting this conclusion.

The writer's results with collodion membranes do not agree with the conclusions of Girard, and we shall see that the solutions of neutral salts act as powerfully on the rate of diffusion of water through collodion membranes as the solutions of alkalies, acids, or acid salts. We

[6] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 39, 237.

shall show that there is no difference between the osmotic effects of the hydroxides and of the chlorides or nitrates, *e.g.* M/128 NaCl and M/128 NaOH acting qualitatively and quantitatively practically alike in their influence on the rate of diffusion of water through collodion membranes.

The writer has carried out a series of experiments on the rate of diffusion of water through a collodion membrane separating the pure solvent from a solution. These experiments indicate that this process depends on two kinds of forces; namely, first, those of gas pressure which are clearly recognizable in the case of solutions of non-conductors like glucose or saccharose, and second, electrical forces which become predominant in the case of low concentrations of solutions of electrolytes. We shall deal in this paper with the electrical forces. They lead to the result that in the diffusion of water through a collodion membrane to a solution of an electrolyte the water molecules are either positively or negatively charged according to the nature of the electrolyte in the solution and that these charges are a factor in the rate of diffusion. The laws or rules controlling this factor can be expressed in the following way.

1. Solutions of neutral salts possessing a univalent or bivalent cation influence the rate of diffusion of water through a collodion membrane, as if the water particles were charged positively and were attracted by the anion and repelled by the cation of the electrolyte; the attractive and repulsive action increasing with the number of charges of the ion and diminishing inversely with a quantity which we will designate arbitrarily as the "radius" of the ion. The same rule applies to solutions of alkalies.

2. Solutions of neutral or acid salts possessing a trivalent or tetravalent cation influence the rate of diffusion of water through a collodion membrane as if the particles of water were charged negatively and were attracted by the cation and repelled by the anion of the electrolyte. Solutions of acids obey the same rule, the high electrostatic effect of the hydrogen ion being probably due to its small "ionic radius."

We shall show first that the assumption of a difference in the sign of the charge of water molecules (according to the two rules just mentioned) explains all the phenomena that can be observed; and

we shall show later that the correctness of the assumption concerning the sign of the charge of the molecules of water can be proved directly by experiments on electric endosmose.

II. Observations on the Rate of Filtration.

Collodion bags in the form of Erlenmeyer flasks of a content of about 50 cc. were prepared in as uniform a way as possible and were used after several days testing. Fresh bags were usually too permeable to serve for our purpose. The mouth of the collodion bag was closed with a perforated rubber stopper and the mouth of the bag was fitted tightly to the stopper with the aid of rubber bands. Through the hole in the stopper a glass tube with a bore of about 2 mm. in diameter was pushed into the bag. The collodion bag was filled with distilled water which reached into the glass tube to a height of about 110 mm. above the level of the distilled water of a beaker into which the bag was dipped. This pressure head of about 110 mm. of water inside the bag increases the number of particles impinging during the unit of time on the unit of area of the inside of the collodion bag and as a consequence more water diffuses in the unit of time from the bag into the beaker than diffuses in the opposite direction. Consequently water diffuses out and the pressure head on the water in the bag diminishes constantly. By measuring the level of the water in the glass tubes at definite intervals and plotting the values of the level as ordinates over the time as abscissæ we get a picture of the rate of diffusion of water out of the bag (Fig. 1).

When the outside solution is not distilled water but a solution of a non-conductor, *e.g.* cane sugar, the rate of diffusion of water is accelerated owing to the fact that the presence of sugar particles diminishes the number of water particles which in the unit of time impinge on the unit of area on the outside of the collodion membrane. Hence if the inside of the bag contains water and if the pressure head of the water inside the bag is at the beginning again about 110 mm. of water, the difference in the number of particles of water impinging on the inside remains the same as before while the number of water molecules impinging on the outside is diminished through the presence of the sugar particles. This diminution must be in proportion to the concentration of the sugar solution.

Fig. 1 gives the curves for the rate of fall in the pressure head of water in the glass tube when the beaker contains distilled water or different molecular concentrations of cane sugar. The curves show that the accelerating influence of a M/64 solution of cane sugar on the rate of outflow of water is still very slight under the conditions of these experiments but that the influence of the sugar solution increases with a further increase in its concentration. These curves may serve as standards of comparison for the curves obtained with

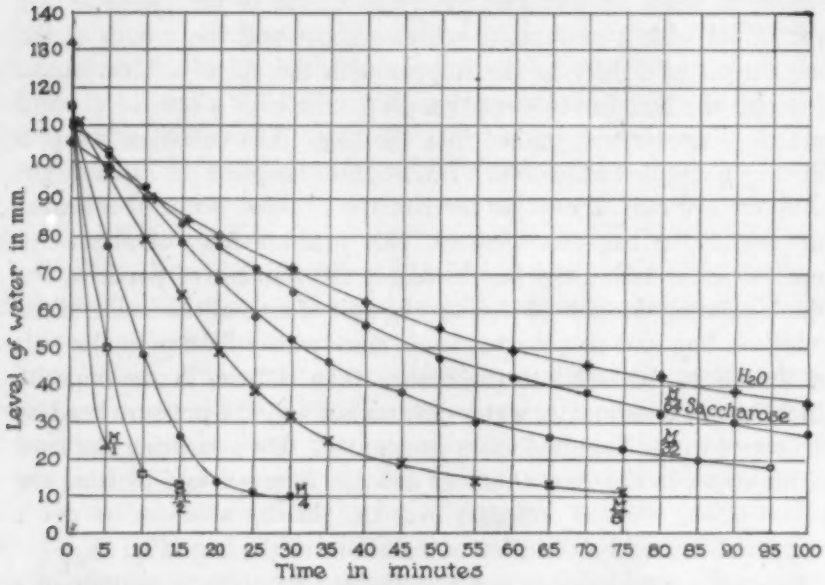


FIG. 1. Curves of fall of level of water diffusing against distilled water and cane sugar solutions of various concentrations.

electrolytes. The influence of solutions of lower concentration of cane sugar than M/64 is not noticeable by this method.

When we replace the sugar solutions in the beaker with solutions of electrolytes of approximately the same osmotic pressure we notice considerable variations in the rate of outflow of water (or rather the fall of the pressure head in the glass tube) with the nature of the electrolyte, and these variations find their expression—as far as the writer's present observations go—in the two rules mentioned above.

Although in these experiments water not only diffuses from the collodion bag into the beaker but electrolyte diffuses also from the beaker into the collodion bag, we shall show in Chapter IV that the differences in our curves are not due to differences in the rate of the diffusion of the electrolytes but to differences in the rate of diffusion of water from the collodion bag into the beaker. Equilibrium will be reached when the solutions inside and outside of the beaker are identical, but our experiments deal only with the initial rate of diffusion of water leading to this condition of equilibrium and not with the condition of equilibrium itself.

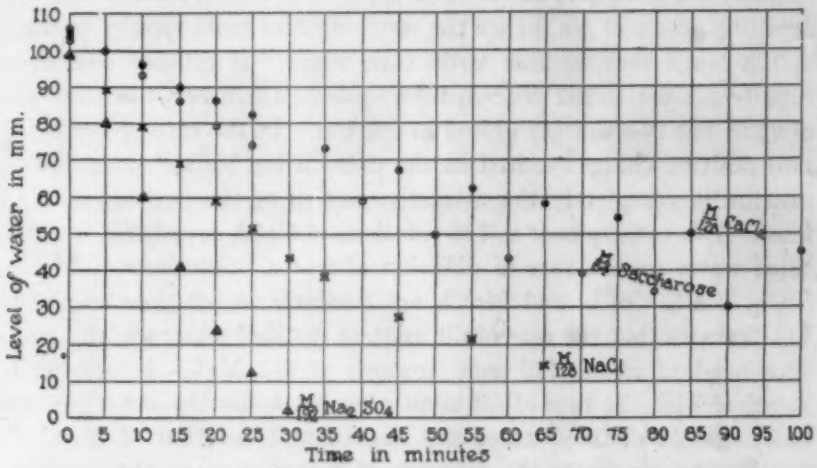


FIG. 2. Curves of fall of level of water when diffusing against solutions of M/64 cane sugar, M/128 $CaCl_2$, M/128 NaCl, and M/192 Na_2SO_4, showing the opposite effect of increase in valency of anion and cation on the rate of diffusion of water.

Fig. 2 illustrates the influence of solutions of three neutral salts, NaCl, $CaCl_2$, and Na_2SO_4, on the rate of diffusion of distilled water from the bag into the beaker containing the salt solution. The water in the bag had an initial pressure head of about 110 mm. of water. Water diffuses more rapidly against a M/128 solution of NaCl and still more rapidly against a M/192 Na_2SO_4 solution than against a M/64 solution of cane sugar. This difference cannot be ascribed to a difference in osmotic pressure of the three solutions since their

osmotic pressure was approximately the same and we shall see later that the phenomenon in question is widely independent of differences in the osmotic pressure of electrolytes. On the other hand, water diffuses more slowly against a M/128 solution of $CaCl_2$ than against a M/64 sugar solution.

These facts may serve as an illustration of our statement that in the case of electrolytes with univalent and bivalent cation water behaves as if it were positively charged and attracted by the anion and repelled by the cation of the salt, and that both attraction and repulsion increase with the number of charges of an ion. In the case of NaCl the attractive action of Cl is for some reason greater than the repelling action of Na, hence the water diffuses more rapidly towards M/128 NaCl than against M/64 cane sugar. It diffuses still more rapidly against M/192 Na_2SO_4 than against M/128 NaCl because the SO_4 ion has two charges united in one ion. In the case of $CaCl_2$ the two positive charges united in the one Ca ion suffice to annihilate practically completely the attractive action of the two negative Cl ions. As a consequence M/128 solutions of $CaCl_2$ act almost like distilled water on the rate of diffusion of water. Solutions of $MgCl_2$, $SrCl_2$, $BaCl_2$, $CoCl_2$, and $MnCl_2$ act similarly to solutions of $CaCl_2$. Fig. 3 shows that the rate of diffusion of distilled water under a pressure head of about 110 mm. towards M/192 $MgCl_2$ is practically identical with the rate of diffusion of water under the same pressure head against distilled water and that a M/4 solution of $MgCl_2$ is required to accelerate the diffusion of water through the collodion membrane to approximately the same amount as is done by a M/128 solution of NaCl.

Fig. 4 shows that the attractive action of anions of salts with univalent cations (Na and K) increases with the valency of the anion. Very dilute solutions of oxalates, phosphates, citrates, and the tetravalent anion $Fe(CN)_6$ attract water so violently that it diffuses extremely rapidly through the membrane. Hence an increase in the number of charges of a cation lowers the attraction of the electrolyte for water, while an increase in the number of charges in an anion increases the attraction. All this agrees with the assumption that the molecules of water possess a positive charge.

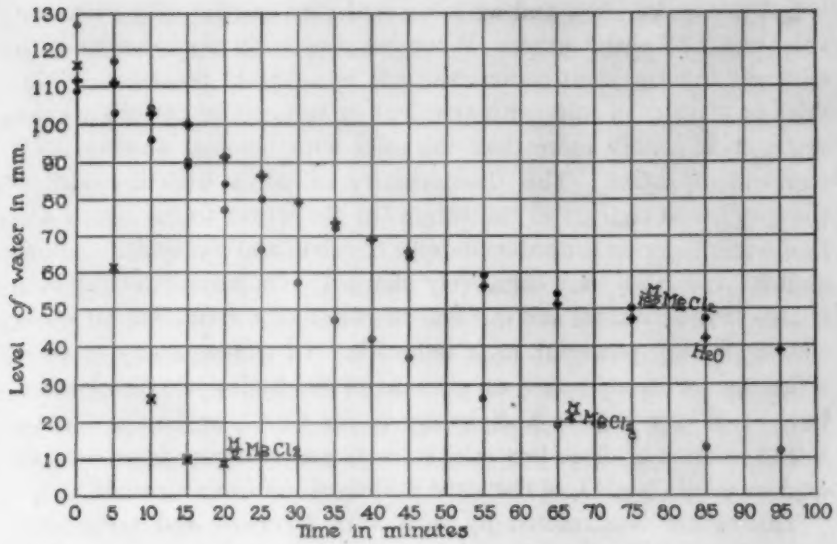


FIG. 3. Curves of fall of level of water when diffusing against solutions of $MgCl_2$ of different concentrations.

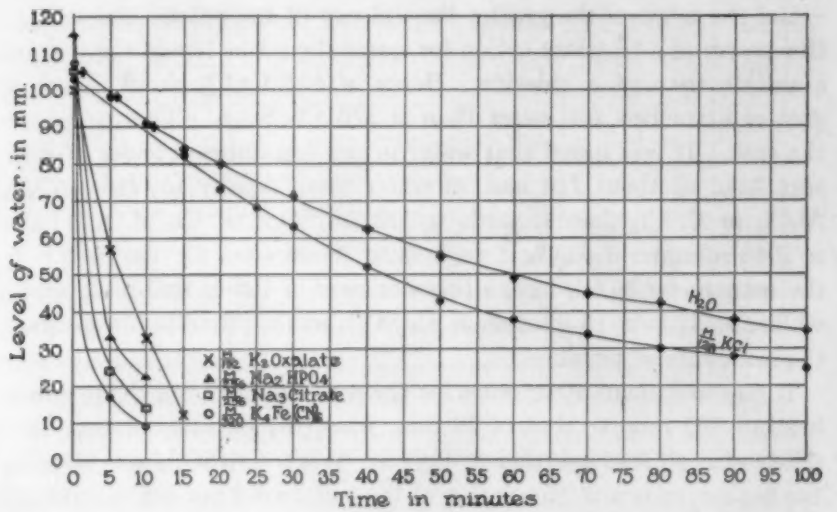


FIG. 4. Curves of fall of level of water when diffusing against solutions of different salts of sodium and potassium, showing the increase in rate with increase in valency of anion.

But this is only true as long as we deal with neutral salts with univalent and bivalent cation. When we deal with neutral salts with trivalent or tetravalent cations the rule is suddenly reversed. Chlorides or nitrates of salts with trivalent or tetravalent cations attract water considerably more than the salts with bivalent or even with monovalent cation. This discontinuity of effect with increase of the number of charges of the cation led the writer to the conclusion that water behaves towards salts with trivalent and tetravalent cations as if its molecules were negatively charged. On account of the high charge of the trivalent and tetravalent cations the attraction for water should be very powerful, as it indeed is. Of course, many of these solutions are strongly acid on account of the hydrolytic dissociation, but this is true only to a slight degree for Ce_2Cl_6 or La_2Cl_6. Yet a M/512 solution of these two salts attracts water almost as powerfully as does M/192 Na_2SO_4 of the same hydrogen ion concentration.

That water is attracted by salts with trivalent and tetravalent cations as if its molecules were negatively charged becomes obvious from a comparison of the effects of the chlorides and sulfates of these salts. Since the anion of a salt should repel negatively charged water —and the more so the greater the valency of the anion—the attractive power of a trivalent cation for water should be less in the case of a sulfate than of a chloride. Hence M/512 Cr_2Cl_6 should have a greater attraction for water than M/320 $Cr_2(SO_4)_3$. This is indeed the case. It was found that water in the bag diffused under a pressure head of about 110 mm. of water more rapidly towards M/512 Al_2Cl_6 or Cr_2Cl_6 than towards M/320 $Al_2(SO_4)_3$ or $Cr_2(SO_4)_3$. In a M/2048 solution of Al_2Cl_6 it took only 20 minutes for the water in the manometer to fall from a pressure head of 105 to that of 10 mm.; while a M/32 to M/16 solution of $Al_2(SO_4)_3$ was required to bring about the same rate of filtration.

It required about 40 minutes for the water to fall from the pressure head of 105 mm. to that of 20 mm. when the outside solution consisted of a M/2048 solution of Cr_2Cl_6. When $Cr_2(SO_4)_3$ was used in the beaker, even a M/16 solution of the sulfate did not act as strongly as a M/2048 solution of the chloride, although the latter was at least partly in suspension. The nitrates of these salts behaved like the chlorides. It is, therefore, obvious that in the case of trivalent cations

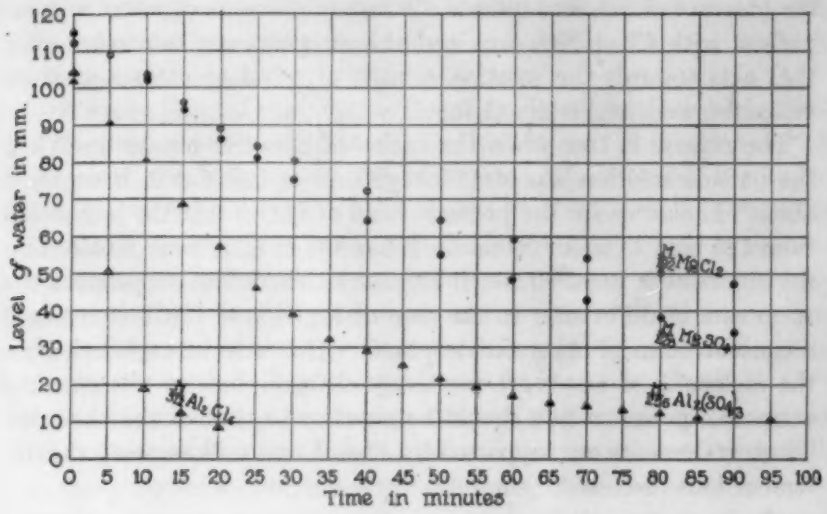
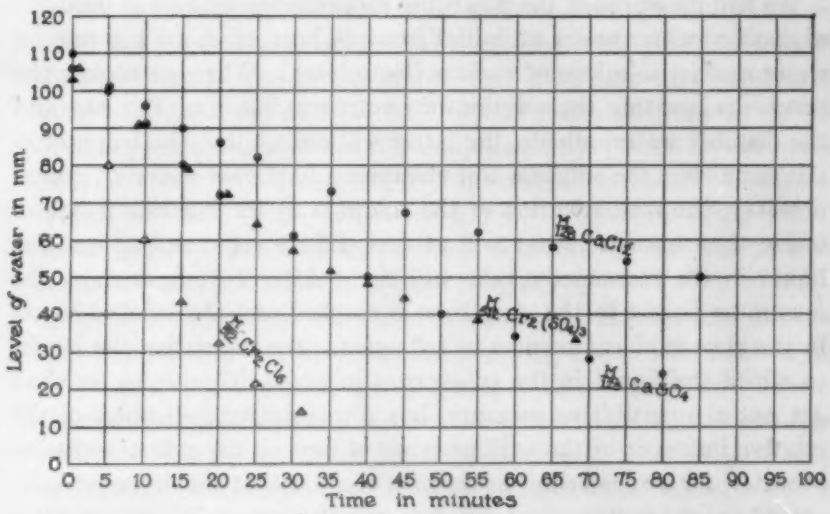


FIG. 5.



FIGS. 5 and 6. Curves showing that in comparison with Cl, SO_4 increases the rate of outflow in the case of bivalent cation and diminishes it in the case of trivalent anion.

the presence of SO_4 ions retards the rate of diffusion of water in comparison with Cl or NO_3 ions and this supports our conclusion that H_2O acts towards the solution of salts of trivalent cations as if its molecules were negatively charged.

The reverse is true when the cation of a salt is bivalent. When the outside solution was M/128 $MgSO_4$ or M/128 $CaSO_4$ it required about 75 minutes for the pressure head of H_2O inside the bag to fall from 120 mm. to about 30 mm. When the sulfates were replaced by the chlorides it required much higher concentrations to produce the same rate of diffusion. In the case of $MgCl_2$ and $CaCl_2$ it required a concentration of approximately M/8. This is to be expected when the molecules of water are positively charged since in this case the attractive power of SO_4 should be superior to that of the chlorides. These statements are supported by Figs. 5 and 6 illustrating the difference here discussed.

III. Modification of the Method.

We had measured in the preceding experiments the rate of diffusion of distilled water under an initial pressure head of about 110 mm. of water against solutions of various electrolytes. When we reverse the procedure, putting the solution of electrolyte inside of the bag and the distilled water outside, the latter will diffuse into the bag and at the same time the solution will commence to diffuse outside. Since, however, the concentration of the solute is at the beginning greater inside than outside, water will at first diffuse into the bag and the liquid in the manometer tube will rise. After 1 hour or more the maximum height in the glass tube is reached and the level of liquid in the glass tube commences to fall again. By comparing the height to which the liquid in the tubes rises in about 70 minutes we shall get not a quantitative measure, but a qualitative indication of the relative influence of the various types of ions on the rate of diffusion of water; for we shall show in the next chapter that the differences observed are the expression of differences in the rate of diffusion of water into the solution and not the expression of differences in the rate of diffusion of the solute into the distilled water. We used M/128 solutions, since we found that M/64 and even M/32 solutions of cane

sugar or dextrose caused only a slight rise in the level of liquid. This excludes the possibility that the rise in liquid observed could be ascribed to the gas pressure of the solute.

A comparison of the relative rise of the liquid in the tube in Table I leads to a verification of the two rules. M/128 solutions of the chlorides of the alkaline metals cause a considerable rise, the rise increasing with an increase in the "radius" of the cation in the order Li, Na, K, Rb (Li having the smallest ionic radius). This agrees with the assumption that water behaves towards these solutions as if its molecules were positively charged and attracted by the anion and repelled by the cation, the repulsion being the greater the smaller the "ionic radius" of the cation.

The most striking fact is that the M/128 solutions of the neutral salts of bivalent metals cause no rise. In fact the water fell constantly in the glass tube containing the solutions, since the initial pressure head in the tube was always about 25 mm. of the column of solution. Hence solutions of M/128 $MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$, $CoCl_2$, and $MnCl_2$ possess actually no attraction for water (beyond that caused by the laws of gas pressure). This was to be expected since the positively charged water molecules are repelled more powerfully by the bivalent than by the univalent cations. This difference between the salts with univalent and bivalent cations exists not only in the case of chlorides but also of nitrates and of sulfates (Table I), and, as we shall see later, the hydroxides. The concentration of all solutions except two used in the table was M/128. The slight differences in the osmotic pressure of the various solutions do not influence the result, as we shall show in a later chapter.

Table II shows that the attractive action of sodium salts increases with the valency of the anion, as our rule demands. Table I also shows that in the case of neutral salts with univalent and bivalent cations the sulfates attract water more powerfully than the chlorides.

Table I shows, moreover, that the salts with trivalent and tetravalent cations have a very powerful attraction for water, which we should expect if water molecules behave like negatively charged bodies towards solutions of these salts.

TABLE I.

A. Solutions which attract water as if its molecules were positively charged.

Chlorides.	Level of solution after 70 min.	Nitrates.	Level of solution after 70 min.	Sulfates.	Level of solution after 70 min.
	mm.		mm.		mm.
LiCl	96	$LiNO_3$	64	Li_2SO_4	356
NaCl	106	$NaNO_3$	112	Na_2SO_4	410
KBr	131				
RbCl	154				
CsCl	143				
$MgCl_2$	12	$Mg(NO_3)_2$	10	$MgSO_4$	74
$CaCl_2$	18	$Ca(NO_3)_2$	22	$CaSO_4$	45
$SrCl_2$	13	$Sr(NO_3)_2$	19		
$BaCl_2$	16	$Ba(NO_3)_2$	15		
$CoCl_2$	15				

B. Solutions which attract water as if its molecules were negatively charged.

Ce_2Cl_6	260	$Ce_2(NO_3)_6$	531	
Al_2Cl_6	376			
Fe_2Cl_6	663			
M/320 $ThCl_4$	630	M/320 $Th(NO_3)_4$	535	

TABLE II.

Solutions which attract water as if its molecules were positively charged.

	Osmotic pressure in mm. of height of solution after 70 min.
	mm.
NaCl	106
$NaNO_3$	112
Na_2SO_4	410
Na_2 succinate	601
Na_2 tartrate	497
$NaHCO_3$	294
Na_2CO_3	520
Na_2HPO_4	484
Na_3 citrate	846
$K_4Fe(CN)_6$	930

IV. The Rate of Diffusion of Electrolytes through the Collodion Membranes.

We therefore see that no rise in the manometer occurs when a collodion bag filled with a M/128 solution of $MgCl_2$ or $CaCl_2$ is dipped into a beaker with distilled water, while a considerable rise occurs when the bag is filled with a M/128 solution of NaCl or Na_2 oxalate. The writer was at first inclined to assume that salts of the type of $MgCl_2$ diffused so rapidly through the collodion membrane that no osmotic effect had time to occur, while the diffusion of NaCl or Na_2 oxalate was supposed to be much slower. In order to test this idea the following experiments were made.

Various concentrations of LiCl, NaCl, KCl, $MgCl_2$, and $CaBr_2$ were put inside of the collodion bag and the latter was surrounded by a beaker containing 350 cc. of H_2O. Every half hour the amount of Cl or Br contained in 20 cc. of the liquid in the beaker was ascertained by titration after Volhard's method. (Table III.)

TABLE III.

Quantity of 0.1 N Cl (or Br) found in 20 cc. of the outside solution.

After 30 minutes.

	M/1	M/2	M/4	M/8	M/16	M/32	M/64
	cc.	cc.	cc.	cc.	cc.	cc.	cc.
LiCl		5.4	2.5	1.28	0.585	0.265	0.140
NaCl		5.5	2.7	1.15	0.635	0.25	0.125
KCl	12.8	5.4	3.2	1.45	0.59	0.32	0.20
$MgCl_2$		10.7	5.7	2.3	1.3	0.7	
$CaBr_2$	23.9	11.8	5.5	2.7	1.05	0.52	

After 60 minutes.

LiCl		8.1	4.1	1.9	0.90	0.425	0.22
NaCl		7.55	4.2	1.75	0.94	0.38	0.195
KCl	15.0	7.6	4.7	2.2	0.95	0.53	0.28
$MgCl_2$	30.4	17.4	9.25	3.6	1.9	1.0	
$CaBr_2$	28.4	14.3	7.3	3.7	1.5	0.8	

These experiments show first that the rate of diffusion of these five salts occurs in proportion to their concentration and second that in the same time almost equal numbers of molecules of LiCl, NaCl, KCl, $MgCl_2$, and $CaBr_2$ diffuse from equally concentrated solutions into the distilled water. (The diffusion velocity of KCl may possibly be slightly larger than that of LiCl or NaCl.)

Hence the fact that the liquid does not rise when the collodion bag is filled with solutions of salts with a bivalent cation while it rises when it is filled with salts with univalent cation of the same osmotic pressure cannot be explained on the assumption that the salts like $MgCl_2$ or $CaCl_2$ diffuse out more rapidly into distilled water than do

TABLE IV.

Quantity of 0.1 N HCl and H_2SO_4 found in 20 cc. of the surrounding H_2O.

After 30 minutes.

	N/1	N/2	N/4	N/8	N/16	N/32	N/64	N/128	N/256	N/312	N/1024
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
HCl	14.9	8.4	4.1	1.76	0.795	0.405	0.175	0.09	0.04	0.02	0.01
H_2SO_4	15.3	7.1	3.3	1.4	0.77	0.33	0.15	0.06	0.02	0.01	0.01

After 60 minutes.

HCl	20.1	11.1	5.5	2.5	1.2	0.54	0.25	0.13	0.07	0.03	0.02
H_2SO_4	18.2	9.4	4.2	2.0	0.99	0.44	0.21	0.10	0.04	0.02	0.01

salts of the type NaCl. The only other possible explanation is that water diffuses more rapidly into a collodion bag filled with a solution of a salt like NaCl than into a bag filled with a salt like $CaCl_2$ of the same osmotic pressure.

A similar experiment was carried out with the rate of diffusion of HCl and H_2SO_4. The results are given in Table IV.

The quantities of acid diffusing out are again for each acid approximately in proportion to the concentration of the acid, and almost equal numbers of molecules diffuse out from the same molecular concentrations of the two acids though the rate of diffusion seems to be a trifle less for H_2SO_4 than for HCl. (The reader will notice that the

equal concentrations in Table IV mean equal normality and not equimolecular concentration as in Table III for the neutral salts.)

In order to compare two salts with univalent and bivalent anions, M/128 NaCl and M/128 Na_2 oxalate were chosen, since they vary considerably in their attraction for water. In the M/128 Na_2 oxalate solution the liquid in the manometer rose in 30 minutes to a height of 420 mm. and in the M/128 solution of NaCl to a height of 60 mm. It might again be argued that this difference was due not to a more rapid diffusion of water into the Na_2 oxalate solution but to a more rapid diffusion of the NaCl into the distilled water. Titration showed that after 30 minutes the NaCl solution inside the bag had been diminished by 3.6 cc. M/100 NaCl per 20 cc. solution, while the Na_2 oxalate solution had been diminished by 3.8 cc. M/100 Na_2 oxalate. Again the difference in the rate of diffusion of the two salts is small and cannot explain the large difference in the osmotic behavior of the two salts. We are therefore compelled to ascribe this difference to a difference in the influence of the ions on the rate of the diffusion of water into these solutions, as the rules expressed at the end of Chapter I demand.

While this is correct for solutions of electrolytes above a molecular concentration of M/128, it is possible that for solutions of electrolytes of a concentration below a certain limit the nature of the electrolyte may modify the result. We intend to deal with this possibility in a later paper.

V. Measurements of the Approximate Value of the Electric Force Influencing Diffusion.

These experiments show that aside from the gas pressure of water a second type of forces, namely electrical forces, influence the rate of diffusion of water from solvent to solution of electrolyte through a collodion membrane. If we denote these two types of forces by p and by e (for the osmotic pressure and the electrical attraction respectively), the question arises whether we cannot arrive at an approximate determination of the value of e. This can be done in a simple way. When we fill the collodion bag with a solution, *e.g.* of M/128 KCl, this solution has approximately the same osmotic

pressure as a M/64 solution of cane sugar. If we surround the bag containing the M/128 solution of KCl with a solution of M/64 cane sugar approximately equal numbers of molecules of water should impinge on both sides of the collodion membrane and no change in the level of the two liquids should occur, if only the osmotic pressure p were active. As a matter of fact the water will diffuse from the M/64 sugar solution into the M/128 solution of KCl owing to the electrical force e due to the electrolyte. By using higher concentrations of cane sugar we arrive at finding one concentration which balances the combined osmotic pressure and electrical force of a M/128 solution of NaCl. The concentration of cane sugar which balances the combined osmotic and electric attraction of M/128 NaCl for water was found to be approximately M/8. Assuming that the osmotic attraction p of M/128 NaCl for water is equal to the osmotic pressure of a M/64 solution of cane sugar, the force e of the M/128 KCl solution must be equal to the osmotic pressure M/8 − M/64 = 7 M/64, or $\frac{7 \times 22.4}{64} = 2.4$ atmospheres. In this way we have determined the value of e for a number of electrolytes by selecting as the balancing concentration that concentration of a cane sugar solution in which the level of liquid does not rise when a beaker filled with the cane sugar solution is dipped into a solution of the electrolyte. This method has the advantage that the concentration of the balancing solution of cane sugar can be roughly estimated in about 5 minutes, although in fact most of our experiments were continued for half an hour or an hour. We used the same set of membranes for a large number of experiments and two sets were used in all; occasionally a membrane began to leak and we had to replace it by another membrane. Measurements of the height of the column of solution inside the tube were taken at first every 5 and later every 10 minutes. The sugar solutions were in the bag and 350 cc. of the M/128 NaCl solution were outside. The initial level of the liquid in the manometer tube was about 25 mm. above the level of the sugar solution. Table V gives the results of our measurements. The balancing solution is always that concentration of cane sugar in which the level of liquid in the manometer tube falls slightly in the first 10 minutes, while in the next higher concentration of cane sugar a marked rise occurs. It

should, however, be stated that this sharp line of demarcation was not found to exist in all solutions. As a consequence the figures of this table serve mainly the purpose of confirming the two rules.

The first vertical column gives the molecular concentration of the solution of the electrolyte which was in every case selected so that on the assumption of complete electrolytic dissociation the solution was isosmotic to a M/64 solution of cane sugar. These solutions were, of course, not strictly isosmotic but we shall see in Chapter VIII that such small differences in osmotic pressure as occurred in these experiments cannot influence the result, on account of the fact that the maximal value of the electric force is generally reached in concentrations of the electrolytes lower than those used in this table and that a further rise in concentration of the electrolyte does not materially affect the value of e. For this reason we must not deduct $\frac{1}{64}$ from the concentration of the balancing solution in order to get the value of e, but a much smaller figure which will fall within the limits of the exactness of our methods. Therefore, the concentration of the balancing solution of cane sugar can be considered as a rough approximation of the value of e.

The second vertical column gives the nature of the electrolyte, the third the pH of the solution, and the fourth the approximate molecular concentration of the *balancing* sugar solution. The fifth column gives the value e in terms of atmospheres of the balancing solution. All the values of the balancing concentration are only rough approximations.

These data find their full explanation on the basis of the two rules expressed at the end of Chapter I.

First we notice that the electrolytes of the first group (A, in Table V) possessing univalent or bivalent cations attract water as if the molecules of the water were positively charged bodies. Thus the concentration of the balancing solution (and the value of e) increases with the increase in the number of charges of the anion and diminishes with an increase in the number of charges of the cation—the latter acting as if it repelled the positively charged water molecules.

We also notice that in Group A the repelling action of the cation increases inversely with its "atomic radius," the lithium salts having a lower balancing concentration and a lower value of e than the sodium or potassium salts with the same anion.

It is especially worth mentioning that the hydroxides act exactly like the chlorides or nitrates, thus disposing of the possibility that the hydroxyl ions occupy a privileged position in the electrification of water.

TABLE V.

Approximate Concentration of Balancing Solution of Cane Sugar for Various Solutions of Electrolytes.

A. Electrolytes towards which water acts as if it were positively charged.

Molecular concentration.	Nature of electrolyte.	pH	Approximate molecular concentration of balancing solution of cane sugar.	Approximate value of ϵ in atmospheres.
M/128	LiCl	5.4	Slightly above M/32	Slightly above 0.7
M/128	NaCl	5.4	M/8	2.8
M/128	KCl	5.3	M/8	2.8
M/128	RbCl	5.5	M/4	5.6
M/192	$MgCl_2$	5.4	M/64	About 0
M/192	$Mg(NO_3)_2$	5.6	Below M/64	" 0
M/192	$CaCl_2$	5.5	" M/64	" 0
M/192	$SrCl_2$	5.4	" M/64	" 0
M/192	$BaCl_2$	5.4	M/64	" 0
M/192	$CoCl_2$	5.0	Below M/64	" 0
M/192	$MnCl_2$	5.3	" M/64	" 0
M/128	LiOH		M/16	1.4
M/128	NaOH		3M/32	2.1
M/128	KOH		M/4	5.6
M/192	$Ca(OH)_2$		Less than M/64	About 0
M/192	$Ba(OH)_2$		" " M/64	" 0
M/128	NH_4OH	10.2	M/32	0.7
M/192	Li_2SO_4	5.4	M/4	5.6
M/192	Na_2SO_4	5.4	Slightly above M/4	Slightly above 5.6
M/192	K_2SO_4	5.4	Less than M/2	" below 11.2
M/128	$MgSO_4$	5.4	M/32	0.7
M/128	$CaSO_4$	5.4	M/16	1.4
M/128	$MnSO_4$	5.4	Slightly less than M/32	Slightly below 0.7
M/128	KCNS	5.5	M/8	2.8
M/128	K acetate	6.3	Slightly above M/4	Slightly above 5.6
M/192	K_2 tartrate	6.4	" " M/2	" " 11.2
M/192	K_2 oxalate	5.9	M/2	11.2
M/256	K_2HPO_4	8.2	Slightly above M/2	Slightly above 11.2
M/256	K_3 citrate	7.8	" " 3M/4	" " 16.8
M/320	$K_4Fe(CN)_6$	6.2	" below 3M/4	" below 16.8

TABLE V—*Concluded.*

B. Electrolytes towards which water acts as if it were negatively charged.

Molecular concentration.	Nature of electrolyte.	pH	Approximate molecular concentration of balancing solution of cane sugar.	Approximate value of ϵ in atmospheres.
M/192	$BeCl_2$.....	4.5	M/8	2.8
M/512	Ce_2Cl_6.....	5.1	M/4	5.6
M/512	La_2Cl_6.....	5.2	Slightly above 3M/8	Slightly above 8.4
M/512	Al_2Cl_6.....	3.8	" " M/2	" " 11.2
M/512	$Cr_2(NO_3)_6$.....		" less than M/2	" below 11.2
M/320	$ThCl_4$.....	3.2	" above 3M/8	" above 8.4
M/128	$BeSO_4$.....	3.5	M/64	0.3
M/320	$Al_2(SO_4)_3$.....	3.8	M/8	2.8
M/320	$Cr_2(SO_4)_3$.....		M/32	0.7
M/128	HCl.....		M/32	0.7
M/128	HNO_3.....	2.1	M/64	0.3
M/128	Acetic acid.....	3.3	Less than M/64	About 0
M/192	H_2SO_4.....		" " M/64	" 0
M/192	Oxalic acid.....	2.2	" " M/64	" 0
M/192	Tartaric acid...	2.6	" " M/64	" 0
M/256	Citric acid.....	3.0	" " M/64	" 0
M/256	H_3PO_4.....	2.4	" " M/64	" 0

The electrolytes of Group *B*, including all salts with trivalent and tetravalent cations, influence the rate of diffusion of water as if the water molecules were negatively charged, being attracted by the cation and repelled by the anion. The correctness of this assumption lies in the fact that the attraction of this group for water is greater when the anion is univalent than when it is bivalent, the repelling action being greater in the latter case. Thus the value for ϵ is in Group *B* greater when the anion is a chloride than when it is a sulfate, while in the preceding group of electrolytes towards which water was positively charged the sulfates acted more strongly than the chlorides or nitrates or hydroxides.

The acids belong to Group *B*, although the hydrogen ion carries only one charge. But the latter fact is compensated by the smallness of the "ionic radius" of hydrogen which is the smallest of all elements. This smallness of the "ionic radius" makes the electrostatic action of the positively charged nucleus comparatively strong. Li comes next among the univalent cations in smallness of "ionic radius"

and this explains why the lithium salts attract water less actively than the sodium salts with the same anion.

Since the solutions of some of the salts with trivalent and tetravalent cations are acid (due to hydrolysis), it might be argued that it is only the presence of free acid which makes the salts of Group *B* behave differently from those of Group *A*. This assumption is disproved by the fact that La_2Cl_6 and Ce_2Cl_6 which belong to Group *B* are neutral salts; *i.e.*, the hydrogen ion concentration of their solution has a pH of the order of that of the solution of neutral salts of Group *A*.

Since, however, the idea of a specific rôle of hydrogen and hydroxyl ions is so general in the colloid literature it seemed of importance to make sure that the differences we have established between the salts

TABLE VI.

Nature of solution.	pH	Concentration of balancing cane sugar solution.
M/512 La_2Cl_6	5.2	Slightly above 3M/8
M/512 Ce_2Cl_6	5.15	" below 3M/8
M/192 $MgCl_2$	5.1	Less than M/64
M/128 $MgSO_4$	5.05	M/64
M/128 KCl	5.0	M/16
M/192 K_2SO_4	5.0	3M/16

of Groups *A* and *B* remain valid when the salts have an identical hydrogen ion concentration. The pH of M/512 La_2Cl_6 was found to be 5.2, that of M/512 Ce_2Cl_6 5.1 (due to the presence of CO_2).[7] We prepared solutions of M/128 KCl, M/192 $MgCl_2$, M/192 K_2SO_4, and M/128 $MgSO_4$ of the same or even a slightly lower pH, namely 5.0 to 5.1. Table VI gives the balancing concentration of cane sugar for the six solutions.

We notice, first, that for the same pH of about 5.1 the salts with bivalent cation show the characteristic low value for *e*, their attraction for water being less than that of the salts of K as well as of La or Ce.

[7] The pH of the distilled water used varied between 5.1 and 5.3 and this accounts for the pH found in our solutions of neutral salts.

We notice, second, that the attraction for water is greater in the case of $MgSO_4$ than in the case of $MgCl_2$, and greater in the case of K_2SO_4 than in the case of KCl, showing that up to pH = 5.0 or 5.1 these salts attract positively charged water molecules. Hence the action of the salts of Group *B* is due to the trivalent or tetravalent cation and the H ion owes its effect not to any specific "adsorption" but to the smallness of its "atomic radius," which gives it a high electrostatic effect.

On the other hand, a high degree of acidity of a solution of a salt with a bivalent cation induces a negative charge in the water molecule. This is true for beryllium salts. In this case the action of H ions is simply added to that of the Be ions (which have a small "ionic ra-

TABLE VII.

Nature of solution.	pH	Concentration of balancing cane sugar solution.
M/192 Na_2CO_3	9.2	Slightly above M/2
M/192 $NaHCO_3$	11.0	" " 3M/8
M/256 Na_3PO_4	12.0	" " M/2
M/256 Na_2HPO_4	8.6	" " M/2
M/256 NaH_2PO_4	4.2	M/16
M/256 H_3PO_4	2.4	Less than M/64

dius"), and the combined effect of both ions exceeds the effect of the Cl ions. We also notice that $BeSO_4$ acts less powerfully than $BeCl_2$, as it should if in the presence of these salts water is negatively charged. The Be salts therefore belong in Group *B*. We shall come back to the effects of a mixture of two electrolytes in another paper.

From what has been said it is to be expected that the substitution of an H for an Na ion in the carbonates or phosphates increases the cation effect owing to the fact that H has a smaller "ionic radius" than Na (Table VII).

It is obvious that in the two carbonates and the first three phosphates toward which (as we shall see in the next chapter) water is positively charged, the attraction of the CO_3 or PO_4 for water diminishes the more the more H ions replace the Na ions, as our theory demands.

VII. Direct Proof of the Influence of the Valency and Radius of Ions on the Sign of the Charge of Water.

Experiments on the migration of water through a collodion membrane under the influence of a constant current (electric endosmose) furnish the direct proof that our assumption is correct.

It is known that if a current is sent through the walls of a porous cell bounded on both sides by the same liquid conductor, water migrates either to the cathode or the anode. This migration is indicated by a rise in the level of the water on the side of that electrode towards which the water migrates. Helmholtz explained this phenomenon by the formation of an electrical double layer at the boundary of the diaphragm and the water particles. When a current is sent through the liquid the positively charged particles of water will move towards the cathode and if the water particles in contact with the wall are negatively charged they will move towards the anode. On account of the internal friction of the liquid the neighboring layers of liquid will be dragged along with the charged particles. This causes the rise of the liquid on the side of that electrode towards which the particles move.[8]

This electric osmose gives us a chance to test our assumption that the water molecules assume a different charge according to the nature of the electrolyte; namely, (1) that the water molecules assume a positive charge when the collodion bag is filled with solutions of neutral salts with a monovalent or bivalent cation or with solutions of hydroxides with monovalent or bivalent cations, (2) that the water molecules assume a negative charge when the collodion bag is filled with solutions of neutral (or acid) salts with a trivalent or tetravalent cation, or with solutions of acids.

If our assumption is correct, water should move towards the cathode in the case of the electrolytes mentioned in (1) (Group *A*, Table V) and towards the anode in the case of electrolytes mentioned in (2) (Group *B*, Table V). This is actually the case.

The collodion bags, closed by a perforated rubber stopper through the hole of which a glass tube with a bore of 2 mm. was inserted,

[8] It should be noticed that a collodion membrane belongs in all probability to the type of non-porous diaphragms.

were filled with a dilute solution of the electrolyte the influence of which on the electrification of water was to be tested. The collodion bag was suspended in a beaker containing the same solution of the electrolyte as the bag, the two solutions being identical in every respect, nature of solute, concentration, and pH. By pushing the glass tube (manometer) a little deeper into the bag the liquid was adjusted so that it rose in the glass tube about 25 mm. above the level in the beaker. One electrode (platinum wire) was dipped into the glass tube, another into the beaker, and a constant current was sent through with an intensity of about 4.8 milliampere. This intensity was kept constant whenever possible. The level of the liquid in the glass tube was measured at the beginning of the experiment and it was easy to determine the sign of the electrification of the water molecules by observing whether the current caused the liquid in the glass tube to rise or to fall. When the anode was in the glass tube and the water rose we could be sure that the water was negatively charged, and when the level of the water fell rapidly under such conditions in the tube, we could be sure that the water was positively charged. When it fell very slowly the fall might have been due to the pressure head in the glass tube. A reversal of the direction of the current removed any doubt that might arise. The experiments gave a complete confirmation of the assumption made in the two rules.

It was found that in the presence of solutions of neutral salts belonging to Group *A* (Table V), *i.e.* salts or hydroxides with univalent cations, the water migrated to the cathode and hence was positively charged, as our theory demands. The following salts were tried: M/1000 and M/500 LiCl, M/1000 and M/500 Li_2SO_4, M/256 NaCl, M/1000 Na_2 oxalate, M/128 Na_2SO_4, M/256 Na acetate, M/256 LiOH, M/256 NaOH, M/1000 KCl, M/512 K_2 oxalate, M/1024 K_3 citrate, and M/1000 $K_4Fe(CN)_6$.

In solutions of the neutral salts with bivalent cation, M/512 $MgCl_2$, $CaCl_2$, $SrCl_2$, and $BaCl_2$, water migrated neither to the anode nor to the cathode; these salts did not cause, or only to a very slight degree, an electrification of the water molecules as was to be expected. M/512 $CaSO_4$ on the other hand, induced a slight migration of water to the cathode, as our theory demanded.

In the presence of electrolytes belonging to Group *B* (Table V) water migrated to the anode, as it should on the basis of our rules. The following electrolytes were tried: M/1024 La_2Cl_6, M/1024 lanthanum ammonium nitrate, M/1024 Ce_2Cl_6, M/1024 Al_2Cl_6, M/1024 $Al_2(SO_4)_3$, and M/1024 $ThCl_4$.

In the presence of M/250 HCl and M/250 HNO_3, tartaric and M/512 phosphoric acids water also migrated to the anode, but the rate of migration was very slow; in the case of M/500 or M/1000 H_2SO_4 practically no migration was noticeable.

In the presence of M/512 $BeCl_2$ water migrated also to the anode as was to be expected. Most interesting were the experiments with M/500 H_3PO_4, NaH_2PO_4, Na_2HPO_4, and Na_3PO_4. All, with the exception of H_3PO_4, caused a migration to the cathode, which again is in agreement with our rule. It may also be stated that when M/512 NaCl solution was rendered acid, *e.g.* when the pH was 4.0 or 3.7, the water migrated to the anode.

We may therefore state that our assumption concerning the influence of electrolytes on the sign of the electrical charge of water molecules is supported not only by the facts for the explanation of which it was made but also by the direct experiments with electrical osmose.

VIII. Influence of the Concentration of the Electrolyte in Solution on the Electric Effect.

When water diffuses from pure solvent to a solution, the initial rate of diffusion seems to vary approximately with the concentration of the solute, as long as the latter is a non-electrolyte. When it is an electrolyte the laws of gas pressure are not suspended but may be masked by the electrical forces which act simultaneously with the laws of gas pressure. It seemed of interest to determine the influence of the concentration of the electrolyte upon the rate of diffusion of water through the collodion membrane. To accomplish this the balancing solution of cane sugar was determined for various concentrations of the electrolyte. Table VIII gives the results of such measurements for various concentrations of KCl, of Na_2SO_4, and of $MgCl_2$.

The effects observed in the solutions of $MgCl_2$ may be only osmotic pressure effects with little or no influence of an electrical character.

In the case of KCl and of Na_2SO_4 it seems that the maximal effect of *e* is reached at a very low concentration of the electrolyte, near M/320 in the case of KCl and near M/960 in the case of Na_2SO_4. A further increase of the concentration to 100 times or more caused no further increase in the value of the balancing concentration. This would be intelligible on the assumption that the electrical effect is of the order of a saturation phenomenon; *e.g.*, the saturation of the membrane with electrolyte. Following ideas suggested in Langmuir's papers,[9] we may assume that electrolyte, collodion, and water ar-

TABLE VIII.

Molecular concentration of KCl.	Concentration of balancing cane sugar solution.	Molecular concentration of Na_2SO_4.	Concentration of balancing cane sugar solution.	Molecular concentration of $MgCl_2$.	Concentration of balancing cane sugar solution.
M/2560	M/64	M/15,360	M/64	M/192	0
M/1280	M/64	M/7680	M/16	10M/192	0
M/960	M/32	M/3840	3M/32	25M/192	0
M/640	M/16	M/1920	M/8	50M/192	Slightly above 3M/32
M/320	M/8	M/960	M/4	100M/192	3M/16
M/128	M/8	10M/192	M/4	150M/192	3M/8
10M/128	M/8	50M/192	3M/16	200M/192	About M/2
100M/128	M/8				

range themselves in the collodion membrane in a definite pattern or space-lattice held together by chemical forces very much as the particles in a crystal, with this difference only that the molecules of water and the ions of the salts in the pattern have the degree o mobility characteristic of liquids. The electric fields created at the two surfaces of the membrane determine the charge of the particles of water impinging upon or lying adjacent to the surface of the membrane. On the solution side the membrane is bounded by the ions of the solute while on the side of pure solvent it is bounded by water alone and this difference determines the direction of the

[9] Langmuir, I., *J. Am. Chem. Soc.*, 1916, xxxviii, 2221.

diffusion under the influence of these forces, which may be electrostatic in character.

It follows from the data of Table VIII that the balancing concentrations given in Table V are the maximal values for e for each of the electrolytes mentioned and that it is not necessary to deduct $\frac{1}{64}$ from this value. The real deduction to be made is so small that it falls within the limits of error of the determination of the balancing concentration and the latter may therefore be taken as the rough expression for the value of the electrical forces.

SUMMARY.

1. When pure water is separated by a collodion membrane from a watery solution of an electrolyte the rate of diffusion of water is influenced not only by the forces of gas pressure but also by electrical forces.

2. Water is in this case attracted by the solute as if the molecules of water were charged electrically, the sign of the charge of the water particles as well as the strength of the attractive force finding expression in the following two rules. (*a*) Solutions of neutral salts possessing a univalent or bivalent cation influence the rate of diffusion of water through a collodion membrane, as if the water particles were charged positively and were attracted by the anion and repelled by the cation of the electrolyte; the attractive and repulsive action increasing with the number of charges of the ion and diminishing inversely with a quantity which we will designate arbitrarily as the "radius" of the ion. The same rule applies to solutions of alkalies. (*b*) Solutions of neutral or acid salts possessing a trivalent or tetravalent cation influence the rate of diffusion of water through a collodion membrane as if the particles of water were charged negatively and were attracted by the cation and repelled by the anion of the electrolyte. Solutions of acids obey the same rule, the high electrostatic effect of the hydrogen ion being probably due to its small "ionic radius."

3. The correctness of the assumption made in these rules concerning the sign of the charge of the water particles is proved by experiments on electrical osmose.

4. A method is given by which the strength of the attractive electric force of electrolytes on the molecules of water can be roughly estimated and the results of these measurements are in agreement with the two rules.

5. The electric attraction of water caused by the electrolyte increases with an increase in the concentration of the electrolyte, but at low concentrations more rapidly than at high concentrations. A tentative explanation for this phenomenon is offered.

6. The rate of diffusion of an electrolyte from a solution to pure solvent through a collodion membrane seems to obey largely the kinetic theory inasmuch as the number of molecules of solute diffusing through the unit of area of the membrane in unit time is (as long as the concentration is not too low) approximately proportional to the concentration of the electrolyte and is the same for the same concentrations of LiCl, NaCl, $MgCl_2$, and $CaCl_2$.

INDEX TO AUTHORS.

B

Bridges, Calvin B. Vermilion-deficiency, 645
—. *See* MORGAN and BRIDGES, 639
Bright, Elizabeth M. *See* REDFIELD and BRIGHT, 255
Boring, Alice M., and **Morgan, T. H.** Lutear cells and hen-feathering, 127
Bovie, W. T., and **Hughes, D. M.** Rate of recovery from the action of fluorite rays, 323
Bovie, W. T., and **Klein, Alice.** Sensitization to heat due to exposure to light of short wavelengths, 331
Brooks, S. C. A theory of the mechanism of disinfection, hemolysis, and similar processes, 61
—, **Matilda Moldenhauer.** Comparative studies on respiration. III. The effect of ether on the respiration and growth of *Bacillus subtilis*, 193

C

Cathcart, P. H. *See* COHN, WOLBACH, HENDERSON, and CATHCART, 221
—. *See* HENDERSON, COHN, CATHCART, WACHMAN, and FENN, 459
Cohn, E. J., Wolbach, S. B., Henderson, L. J., and **Cathcart, P. H.** On the control of rope in bread, 221
—. *See* HENDERSON, COHN, CATHCART, WACHMAN, and FENN, 459
Cohn, E. J. *See* HENDERSON, FENN, and COHN, 387
Crozier, W. J. Intracellular acidity in *Valonia*, 581
—. On the control of the response to shading in the branchiæ of *Chromodoris*, 585

F

Fenn, W. O. *See* HENDERSON, COHN, CATHCART, WACHMAN, and FENN, 459
—. *See* HENDERSON, FENN, and COHN, 387
Fred, E. B. The growth of higher plants in soils free of microorganisms, 623
— and **Haas, A. R. C.** The etching of marble by roots in the presence and absence of bacteria, 631

G

Garrey, Walter E. Light and the muscle tonus of insects. The heliotropic mechanism, 101
Gustafson, F. G. Comparative studies on respiration. II. The effect of anesthetics and other substances on the respiration of *Aspergillus niger*, 181

H

Haas, A. R. C. *See* FRED and HAAS, 631
—. *See* OSTERHOUT and HAAS, 1, 295
Hackh, Ingo W. D. Bioelements; the chemical elements of living matter, 429

Harvey, E. Newton. Studies on bioluminescence. VII. Reversibility of the photogenic reaction in *Cypridina*, 133
—. IX. Chemical nature of *Cypridina* luciferin and *Cypridina* luciferase, 269
—. The relation between the oxygen concentration and rate of reduction of methylene blue by milk, 415
Hecht, Selig. Sensory equilibrium and dark adaptation in *Mya arenaria*, 545
—. The effect of temperature on the latent period in the photic response of *Mya arenaria*, 667
—. The nature of the latent period in the photic response of *Mya arenaria*, 657
—. The photic sensitivity of *Ciona intestinalis*, 147
Henderson, L. J., Cohn, Edwin J., Cathcart, P. H., Wachman, J. D. and **Fenn, W. O.** A study of the action of acid and alkali on gluten, 459
—, **Fenn, W. O.,** and **Cohn, Edwin J.** Influence of electrolytes upon the viscosity of dough, 387
—. *See* COHN, WOLBACH, HENDERSON, and CATHCART, 221
Hughes, D. M. *See* BOVIE and HUGHES, 323

I

Irwin, Marian. Comparative studies on respiration. V. The effect of ether on the production of carbon dioxide by animals, 209
—. VI. Increased production of carbon dioxide accompanied by decrease of acidity, 399
Itano, Arao, and **Neill, James.** Influence of temperature and hydrogen ion concentration upon the spore cycle of *Bacillus subtilis*, 421

K

Klein, Alice. *See* BOVIE and KLEIN, 331

L

Loeb, Jacques. Amphoteric colloids. I. Chemical influence of the hydrogen ion concentration, 39
—. II. Volumetric analysis of ion-protein compounds; the significance of the isoelectric point for the purification of amphoteric colloids, 237
—. III. Chemical basis of the influence of acid upon the physical properties of gelatin, 363
—. IV. The influence of the valency of cations upon the physical properties of gelatin, 483
—. V. The influence of the valency of anions upon the physical properties of gelatin, 559
—. The influence of electrolytes on the electrification and the rate of diffusion of water through collodion membranes, 717
—. The law controlling the quantity of regeneration in the stem of *Bryophyllum calycinum*, 81
—. The physiological basis of morphological polarity in regeneration. I, 337
—. The physiological basis of morphological polarity in regeneration. II, 687

M

McClure, Charles F. W. On the experimental production of edema in larval and adult Anura, 261
Moore, A. R. Reversal of reaction by means of strychnine in planarians and starfish, 97
—. The respiratory rate of the sciatic nerve of the frog in rest and activity, 613

Moore, A. R. The selective action of nicotine on the central nervous system of the squid, *Loligo pealii*, 505

Morgan, T. H., and **Bridges, Calvin B.** The inheritance of a fluctuating character, 639

—. *See* BORING and MORGAN, 127

N

Neill, James. *See* ITANO and NEILL, 421

Northrop, J. H. The effect of various acids on the digestion of proteins by pepsin, 607

du Noüy, P. Lecomte. A new apparatus for measuring surface tension, 521

O

Osterhout, W. J. V. A comparative study of permeability in plants, 299

—. A comparison of permeability in plant and animal cells, 409

—. A method of studying respiration, 17

—. An indicator method of measuring the consumption of oxygen, 167

—. Antagonism between alkaloids and salts in relation to permeability, 515

—. Comparative studies on respiration. I. Introduction, 171

—. Decrease of permeability and antagonistic effects caused by bile salts, 405

—, and **Haas, A. R. C.** On the dynamics of photosynthesis, 1

—, and —. The temperature coefficient of photosynthesis, 295

P

Packard, Charles. Difference in the action of radium on green plants in the presence and absence of light, 37

Parker, G. H. The rate of transmission in the nerve net of the cœlenterates, 231

Patten, Bradley M. Photoreactions of partially blinded whiptail scorpions, 435

R

Redfield, Alfred C., and **Bright, Elizabeth M.** Temperature coefficient of the action of β-rays upon the egg of *Nereis*, 255

S

Swingle, W. W. Iodine and the thyroid. III. The specific action of iodine in accelerating amphibian metamorphosis, 593

—. On the experimental production of edema by nephrectomy, 509

T

Thomas, Helen Stillwell. Comparative studies on respiration. IV. The effect of ether on the respiration of wheat, 203

U

Uhlenhuth, Eduard. Further proof of the existence of a specific tetany-producing substance in the thymus gland, 33

—. Nature of the retarding influence of the thymus upon amphibian metamorphosis, 305

—. Parathyroids and calcium metabolism, 315

—. Relation between metamorphosis and other developmental phenomena in amphibians, 525

Uhlenhuth, Eduard. Relation between thyroid gland, metamorphosis, and growth, 473

—. The antagonism between thymus and parathyroid glands, 23

W

Wachman, J. D. *See* HENDERSON, COHN, CATHCART, WACHMAN, and FENN, 459

Wolbach, S. B. *See* COHN, WOLBACH, HENDERSON, and CATHCART, 221

INDEX TO SUBJECTS.

A

Acid:
Gelatin, physical properties, influence on, of (LOEB) 363
Gluten, action of acid on (HENDERSON, COHN, CATHCART, WACHMAN, and FENN) 459
Protein digestion by pepsin, effect on, of (NORTHROP) 607

Acidity:
Decrease, increased production of carbon dioxide accompanied by (IRWIN) 399
Intracellular, in *Valonia* (CROZIER) 581

Activity:
Sciatic nerve of frog, respiratory rate (MOORE) 613

Adaptation:
Dark, in *Mya arenaria* (HECHT) 545

Alkali:
Gluten, action of alkali on (HENDERSON, COHN, CATHCART, WACHMAN, and FENN) 459

Alkaloids:
Antagonism to salts in relation to permeability (OSTERHOUT) 515

Amphibian:
Metamorphosis and developmental phenomena (UHLENHUTH) 525
—, specific action of iodine in accelerating (SWINGLE) 593

Analysis:
Volumetric, of ion-protein compounds (LOEB) 237

Anesthetic:
Aspergillus niger, respiration, effect on, of (GUSTAFSON) 181

Animal:
Cells, permeability (OSTERHOUT) 409

Anion:
Valency, influence upon physical properties of gelatin (LOEB) 559

Antagonism:
Alkaloid and salt, in relation to permeability (OSTERHOUT) 515
Bile salts, antagonistic effects (OSTERHOUT) 405
Thymus and parathyroid glands (UHLENHUTH) 23

Anura:
Adult, experimental production of edema (McCLURE) 261
Larval, experimental production of edema (McCLURE) 261

Apparatus:
Surface tension, measuring (DU NOÜY) 521

Arenaria:
Mya, dark adaptation (HECHT) 545
—, latent period in photic response, effect of temperature (HECHT) 667
—, sensory equilibrium (HECHT) 545

Aspergillus:
niger, respiration, effect of anesthetics (GUSTAFSON) 181

B

Bacillus:
subtilis, respiration, effect of ether (BROOKS) 193
—, spore cycle, influence of hydrogen ion concentration (ITANO and NEILL) 421
—, — —, influence of temperature (ITANO and NEILL) 421
Bacteria:
Marble etched by roots in absence of (FRED and HAAS) 631
— — — — in presence of (FRED and HAAS) 631
Bile:
Salts, decrease of permeability and antagonistic effects caused by (OSTERHOUT) 405
Bioelements:
(HACKH) 429
Bioluminescence:
(HARVEY) 133, 269
Branchiæ:
Chromodoris, control of response to shading (CROZIER) 585
Bread:
Rope, control, (COHN, WOLBACH, HENDERSON, and CATHCART) 221
Bryophyllum:
calycinum, regeneration in stem (LOEB) 81

C

Calcium:
Metabolism (UHLENHUTH) 315
Calycinum:
Bryophyllum, regeneration in stem (LOEB) 81
Carbon dioxide:
Production by animals, effect of ether (IRWIN) 209
—, increased, accompanied by decrease of acidity (IRWIN) 399
Cations:
Valency, influence upon physical properties of gelatin (LOEB) 483
Cell:
Animal, permeability (OSTERHOUT) 409
Lutear (BORING and MORGAN) 127
Plant, permeability (OSTERHOUT) 409
Character:
Fluctuating, inheritance (MORGAN and BRIDGES) 639
Chromodoris:
Branchiæ, control of response to shading (CROZIER) 585
Ciona:
intestinalis, photic sensitivity (HECHT) 147
Coefficient:
Temperature of action of β-rays upon eggs of *Nereis* (REDFIELD and BRIGHT) 255
—, of photosynthesis (OSTERHOUT and HAAS) 295
Cœlenterates:
Nerve net, rate of transmission (PARKER) 231
Colloids:
Amphoteric (LOEB) 39, 237, 363, 483, 559
—, significance of isoelectric point for purification (LOEB) 237

Collodion:
Membranes, influence of electrolytes on rate of diffusion of water through (LOEB) 717

Concentration:
Oxygen, relation between, and rate of reduction of methylene blue by milk (HARVEY) 415

Cycle:
Spore, of *Bacillus subtilis*, influence of hydrogen ion concentration (ITANO and NEILL) 421
—, of *Bacillus subtilis*, influence of temperature (ITANO and NEILL) 421

Cypridina:
Luciferase, chemical nature (HARVEY) 269
Luciferin, chemical nature (HARVEY) 269
Reversibility of photogenic reaction (HARVEY) 133

D

Deficiency:
Vermilion- (BRIDGES) 645

Development:
Amphibians, developmental phenomena in (UHLENHUTH) 525

Diffusion:
Rate, of water, through collodion membranes, influence of electrolytes (LOEB) 717

Digestion:
Protein, by pepsin, effect of acids (NORTHROP) 607

Disinfection:
Mechanism (BROOKS) 61

Dough:
Viscosity, influence of electrolytes (HENDERSON, FENN, and COHN) 387

Dynamics:
Photosynthesis (OSTERHOUT and HAAS) 1

E

Edema:
Anura, adult, experimental (McCLURE) 261
—, larval, experimental (McCLURE) 261
Production by nephrectomy (SWINGLE) 509

Egg:
Nereis, temperature coefficient of action of β-rays (REDFIELD and BRIGHT) 255

Electrification:
Water, influence of electrolytes (LOEB) 717

Electrolyte:
Diffusion of water, rate, through collodion membranes, influence on, of (LOEB) 717
Dough viscosity, influence on, of (HENDERSON, FENN, and COHN) 387
Electrification of water, influence on, of (LOEB) 717

Equilibrium:
Sensory, in *Mya arenaria* (HECHT) 545

Etching:
Marble, by roots in absence of bacteria (FRED and HAAS) 631
—, by roots in presence of bacteria (FRED and HAAS) 631

Ether:
Bacillus subtilis, growth, effect on, of (BROOKS) 193
— —, respiration, effect on, of (BROOKS) 193
Carbon dioxide production by animals, effect on, of (IRWIN) 209
Wheat, respiration, effect on, of (THOMAS) 203

Exposure:
Light of short wave-lengths, sensitization to heat due to (BOVIE and KLEIN) 331

F

Feathering:
Hen- (BORING and MORGAN) 127
Fluorite:
Rays, rate of recovery from action of (BOVIE and HUGHES) 323

G

Gelatin:
Physical properties, influence of acid (LOEB) 363
— —, influence of valency of anions (LOEB) 559
— —, influence of valency of cations (LOEB) 483
Gland:
Parathyroid, antagonism between thymus and (UHLENHUTH) 23
Thymus, antagonism between parathyroid and (UHLENHUTH) 23
—, specific tetany-producing substance in (UHLENHUTH) 33
Thyroid, relation to metamorphosis and growth (UHLENHUTH) 473
Gluten:
Acid, action on (HENDERSON, COHN, CATHCART, WACHMAN, and FENN) 459
Alkali, action on (HENDERSON, COHN, CATHCART, WACHMAN, and FENN) 459
Growth:
Bacillus subtilis, effect of ether (BROOKS) 193
Plants, in soils free of microorganisms (FRED) 623
Growth—*continued:*
Thyroid, relation to metamorphosis and (UHLENHUTH) 473

H

Heat:
Sensitization, due to exposure to light of short wave-lengths (BOVIE and KLEIN) 331
Heliotropism:
Mechanism (GARREY) 101
Hemolysis:
Mechanism (BROOKS) 61
Hen:
-feathering (BORING and MORGAN) 127
Hydrogen ion:
Concentration, chemical influence (LOEB) 39
—, influence upon spore cycle of *Bacillus subtilis* (ITANO and NEILL) 421

I

Indicator:
Method of measuring oxygen consumption (OSTERHOUT) 167
Inheritance:
Character, fluctuating (MORGAN and BRIDGES) 639
Insect:
Muscle tonus (GARREY) 101
Intestinalis:
Ciona, photic sensitivity (HECHT) 147
Iodine:
(SWINGLE) 593
Amphibian metamorphosis, specific action of iodine in accelerating (SWINGLE) 593
Ion-protein:
Compounds, volumetric analysis (LOEB) 237

Isoelectric point:
Amphoteric colloids, significance of isoelectric point for purification (LOEB) 237

L

Light:
(GARREY) 101
Plants, green, action of radium on, in absence of (PACKARD) 37
—, —, action of radium on, in presence of (PACKARD) 37
Sensitization to heat due to exposure to light of short wave-lengths (BOVIE and KLEIN) 331
Loligo:
pealii, central nervous system, selective action of nicotine on (MOORE) 505
Luciferase:
Cypridina, chemical nature (HARVEY) 269
Luciferin:
Cypridina, chemical nature (HARVEY) 269
Lutear cell:
See Cell.

M

Marble:
Etching, by roots in absence of bacteria (FRED and HAAS) 631
—, by roots in presence of bacteria (FRED and HAAS) 631
Matter:
Living, chemical elements (HACKH) 429
Membrane:
Collodion, rate of diffusion of water through, influence of electrolytes (LOEB) 717
Metabolism:
Calcium (UHLENHUTH) 315
Metamorphosis:
Amphibian (UHLENHUTH) 525
—, retarding influence of thymus (UHLENHUTH) 305
—, specific action of iodine in accelerating (SWINGLE) 593
Thyroid, relation to growth and (UHLENHUTH) 473
Methylene blue:
Reduction, by milk, rate (HARVEY) 415
Microorganism:
Growth of higher plants in soils free of (FRED) 623
Milk:
Methylene blue reduced by, relation to oxygen concentration (HARVEY) 415
Morphology:
Polarity, morphological, in regeneration, physiological basis (LOEB) 337, 687
Muscle:
Tonus, of insects (GARREY) 101
Mya:
arenaria, dark adaptation (HECHT) 545
—, latent period in photic response, effect of temperature (HECHT) 667
—, sensory equilibrium (HECHT) 545

N

Nephrectomy:
Edema produced by (SWINGLE) 509
Nereis:
Egg, temperature coefficient of action of β-rays (REDFIELD and BRIGHT) 255

Nerve:
Sciatic, of frog, respiratory rate in activity (MOORE) 613
—, — —, respiratory rate in rest (MOORE) 613
Nerve net:
Cœlenterates, rate of transmission in (PARKER) 231
Nervous system:
Central, of squid, selective action of nicotine on (MOORE) 505
Nicotine:
Squid, central nervous system, selective action on, of (MOORE) 505
Niger:
Aspergillus, respiration, effect of anesthetics (GUSTAFSON) 181

O

Oxygen:
Concentration, relation between, and rate of reduction of methylene blue by milk (HARVEY) 415
Consumption, indicator method of measuring (OSTERHOUT) 167

P

Parathyroid:
(UHLENHUTH) 315
Antagonism between thymus and (UHLENHUTH) 23
Pealii:
Loligo, central nervous system, selective action of nicotine on (MOORE) 505
Permeability:
Animal cells (OSTERHOUT) 409
Antagonism between alkaloids and salts in relation to (OSTERHOUT) 515
Permeability—*continued:*
Decrease, caused by bile salts (OSTERHOUT) 405
Plant (OSTERHOUT) 299
— cells (OSTERHOUT) 409
Photic:
Response, of *Mya arenaria*, effect of temperature on latent period (HECHT) 667
—, of *Mya arenaria*, latent period (HECHT) 657
Sensitivity, of *Ciona intestinalis* (HECHT) 147
Photogenic:
Reaction, in *Cypridina*, reversibility (HARVEY) 133
Photoreaction:
Scorpions, whip-tail, partially blinded (PATTEN) 435
Photosynthesis:
Dynamics (OSTERHOUT and HAAS) 1
Temperature coefficient (OSTERHOUT and HAAS) 295
Physiology:
Polarity, morphological, in regeneration, physiological basis (LOEB) 337, 687
Planarian:
Strychnine, reversal of reaction by, in (MOORE) 97
Plant:
Cells, permeability (OSTERHOUT) 409
Green, action of radium on, in absence of light (PACKARD) 37
—, action of radium on, in presence of light (PACKARD) 37
Growth in soils free of microorganisms (FRED) 623
Permeability (OSTERHOUT) 299

Polarity:
Morphological, physiological basis of, in regeneration (LOEB) 337, 687
Protein:
Digestion, by pepsin, effect of acids (NORTHROP) 607
Purification:
Amphoteric colloids, significance of isoelectric point (LOEB) 237

R

Radium:
Green plants in absence of light, action on, of (PACKARD) 37
— — in presence of light, action on, of (PACKARD) 37
Ray:
Fluorite, rate of recovery from action of (BOVIE and HUGHES) 323
β-ray:
Temperature coefficient of action upon egg of *Nereis* (REDFIELD and BRIGHT) 255
Reaction:
Planarians, reversal, by strychnine, (MOORE) 97
Photogenic, reversibility of, in *Cypridina* (HARVEY) 133
Starfish, reversal, by strychnine, (MOORE) 97
Recovery:
Fluorite rays, action of, rate of recovery (BOVIE and HUGHES) 323
Reduction:
Methylene blue, rate of, by milk, relation of oxygen concentration (HARVEY) 415
Regeneration:
Bryophyllum calycinum, stem (LOEB) 81
Physiological basis of morphological polarity (LOEB) 337, 687
Respiration:
(BROOKS) 193
(GUSTAFSON) 181
(IRWIN) 209, 399
(OSTERHOUT) 171
(THOMAS) 203
Aspergillus niger, effect of anesthetics (GUSTAFSON) 181
Bacillus subtilis, effect of ether (BROOKS) 193
Method of studying (OSTERHOUT) 17
Sciatic nerve of frog, rate in activity (MOORE) 613
— — of frog, rate in rest (MOORE) 613
Wheat, effect of ether (THOMAS) 203
Response:
Branchiæ of *Chromodoris*, control of response to shading (CROZIER) 585
Photic, of *Mya arenaria*, effect of temperature on latent period (HECHT) 667
—, of *Mya arenaria*, latent period (HECHT) 657
Rest:
Sciatic nerve of frog, respiratory rate (MOORE) 613
Root:
Marble etched by, in absence of bacteria (FRED and HAAS) 631
—, — —, in presence of bacteria (FRED and HAAS) 631
Rope:
Bread control (COHN, WOLBACH, HENDERSON, and CATHCART) 221

S

Salt(s):
Antagonism to alkaloids in relation to permeability (OSTERHOUT) 515
Bile, decrease of permeability and antagonistic effects caused by (OSTERHOUT) 405

Sciatic:
Nerve, of frog, respiratory rate in activity (MOORE) 613
—, — —, respiratory rate in rest (MOORE) 613

Scorpions:
Whip-tail, partially blinded, photoreactions (PATTEN) 435

Sensitivity:
Photic, of *Ciona intestinalis* (HECHT) 147

Sensitization:
Heat, due to exposure to light of short wave-lengths (BOVIE and KLEIN) 331

Shading:
Branchiæ of *Chromodoris*, control of response to (CROZIER) 585

Soil:
Plant growth in soil free of microorganisms (FRED) 623

Spore:
Cycle, *Bacillus subtilis*, influence of hydrogen ion concentration (ITANO and NEILL) 421
—, — —, influence of temperature (ITANO and NEILL) 421

Squid:
Central nervous system, selective action of nicotine on (MOORE) 505

Starfish:
Strychnine, reversal of reaction by, in (MOORE) 97

Stem:
Bryophyllum calycinum, regeneration (LOEB) 81

Strychnine:
Planarians, reversal of reaction in, by (MOORE) 97
Starfish, reversal of reaction in, by (MOORE) 97

Substance:
Tetany-producing, specific, in thymus gland (UHLENHUTH) 33

Subtilis:
Bacillus, spore cycle, influence of hydrogen ion concentration (ITANO and NEILL) 421
—, — —, influence of temperature (ITANO and NEILL) 421

Surface tension:
Apparatus for measuring (DU NOÜY) 521

System:
Central nervous, of squid, selective action of nicotine on (MOORE) 505

T

Temperature:
Bacillus subtilis, spore cycle, influence on, of (ITANO and NEILL) 421
Coefficient of action of β-rays upon egg of *Nereis* (REDFIELD and BRIGHT) 255
—, of photosynthesis (OSTERHOUT and HAAS) 295
Mya arenaria, latent period in photic response, effect on, of (HECHT) 667

Tetany:
-producing substance, specific, in thymus gland (UHLENHUTH) 33

Thymus:
Amphibian metamorphosis, retarding influence of (UHLENHUTH) 305
Antagonism between parathyroid and (UHLENHUTH) 23
Gland, antagonism (UHLENHUTH) 23
—, specific tetany-producing substance in (UHLENHUTH) 33
Thyroid:
(SWINGLE) 593
Metamorphosis and growth, relation between thyroid and (UHLENHUTH) 473
Tonus:
Muscle, of insects (GARREY) 101
Transmission:
Rate, in nerve net of cœlenterates (PARKER) 231

V

Valency:
Anion, influence upon physical properties of gelatin (LOEB) 559
Valency—*continued:*
Cation, influence upon physical properties of gelatin (LOEB) 483
Valonia:
Acidity, intracellular (CROZIER) 581
Vermilion:
-deficiency (BRIDGES) 645
Viscosity:
Dough, influence of electrolytes (HENDERSON, FENN, and COHN) 387

W

Water:
Diffusion, rate, through collodion membranes, influence of electrolytes (LOEB) 717
Wave-length:
Short, sensitization to heat due to exposure to light of (BOVIE and KLEIN) 331
Wheat:
Respiration, effect of ether (THOMAS) 203

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CONTENTS

CROZIER, W. J. Intracellular acidity in *Valonia* 581

CROZIER, W. J. On the control of the response to shading in the branchiæ of *Chromodoris* 585

SWINGLE, W. W. Iodine and the thyroid. III. The specific action of iodine in accelerating amphibian metamorphosis 593

NORTHROP, J. H. The effect of various acids on the digestion of proteins by pepsin 607

MOORE, A. R. The respiratory rate of the sciatic nerve of the frog in rest and activity 613

FRED, E. B. The growth of higher plants in soils free of micro-organisms 623

FRED, E. B., and HAAS, A. R. C. The etching of marble by roots in the presence and absence of bacteria 631

MORGAN, T. H., and BRIDGES, CALVIN B. The inheritance of a fluctuating character 639

BRIDGES, CALVIN B. Vermilion-deficiency 645

HECHT, SELIG. The nature of the latent period in the photic response of *Mya arenaria* 657

HECHT, SELIG. The effect of temperature on the latent period in the photic response of *Mya arenaria* 667

LOEB, JACQUES. The physiological basis of morphological polarity in regeneration. II 687

LOEB, JACQUES. The influence of electrolytes on the electrification and the rate of diffusion of water through collodion membranes. 717

Index to Volume I 747

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